UNIVERSITY OF MICHIGAN
APR 16 1956
MEDICAL LIBRARY
DO[illegible] NOT CIRCULATE

VOLUME 98 NUMBER 3

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

March, 1956

Carbonic Anhydrase Inhibition. IV: The Effects of Metabolic Acidosis on the Response to Diamox®.
THOMAS H. MAREN 159

Hereditary Elliptocytosis and Hemoglobin C Trait. A Report of Two Cases.
MARY ELLEN AVERY 184

The Effect of Hydrocortisone upon the Course of Pneumococcal Pneumonia Treated with Penicillin.
HENRY N. WAGNER, JR., IVAN L. BENNETT, JR., LOUIS LASAGNA, LEIGHTON E. CLUFF, MIRIAM B. ROSENTHAL AND GEORGE S. MIRICK 197

Studies on the Pathogenesis of Fever. V. The Fever Accompanying Pneumococcal In´ection in the Rabbit. IVAN L. BENNETT, JR. 216

Abstracts of Papers Presented Before the Meeting of The Johns Hopkins Medical Society. December 12, 1955 236

Book Reviews 240

PUBLISHED MONTHLY
THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland
Made in United States of America

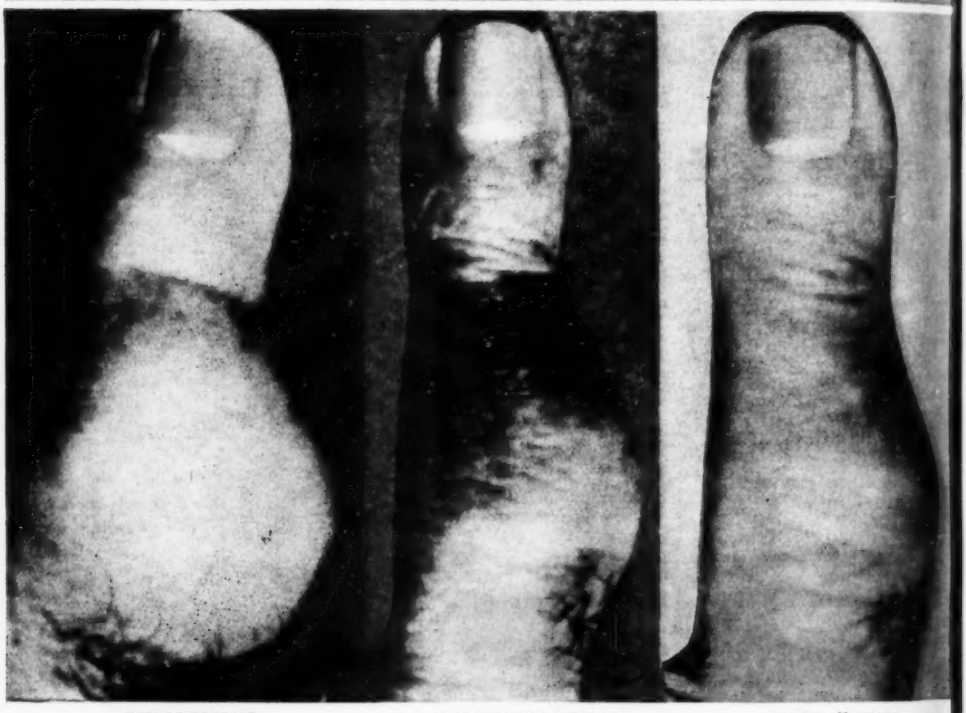
COPYRIGHT, 1956, BY THE JOHNS HOPKINS PRESS

Benemid®

PROBENECID

greatly benefits chronic gout

MAJOR ADVANTAGES: Markedly reduces old tophi — helps prevent new ones. Diminishes intercritical symptoms. Decreases frequency and severity of acute attacks.



BEFORE BENEMID | WITH BENEMID 2 YEARS | AFTER 2½ YEARS

Prolonged therapy with BENEMID produces dramatic results in patients crippled by chronic gouty arthritis. Investigators[1] report bedridden cases able to walk again... patients with stiff and painful joints returning to work, even when this requires skilled use of the hands.

An exceptionally well-tolerated uricosuric agent,[1] BENEMID restores and maintains normal serum uric acid levels.[2] Thus urate deposits in the joints are reduced, new ones prevented. Prolonged therapy "may reduce the need for surg interference."[3] Toxic reactions are unusual.

Supplied: 0.5 *Gm.* tablets. *Dosage* 1 to 4 tab

Note: To maintain or secure full uricos effect, salicylates should not be administe concurrently with BENEMID.

References: 1. J.A.M.A. 149:1188, 1952. 2. Ann. Int. Med. 42:9, 1955. 3. Gout and Gouty Arthritis, Modern Medical Monograph, 7, New York, Grune & Stratton, 1953, p. 80.

SHARP & DOHME

Philadelphia 1, Pa.
DIVISION OF MERCK & CO., INC.

ew ones.
attacks.

ARS

d for surg
unusual.
1 to 4 tabl
ll uricos
administe

INC.

CARBONIC ANHYDRASE INHIBITION. IV: THE EFFECTS OF METABOLIC ACIDOSIS ON THE RESPONSE TO DIAMOX®[1]

THOMAS H. MAREN[2]

Experimental Therapeutics Section, Research Division, American Cyanamid Company, Stamford, Connecticut

Received for publication October 10, 1955

TABLE OF CONTENTS

	Page
Introduction	159
Methods and Materials	160
Experimental	
A. Continuous Carbonic Anhydrase Inhibition	162
1. Renal Excretion	163
(a) HCO_3^- Excretion	163
(b) Na^+ Excretion	164
(c) K^+ Excretion	165
2. Metabolic Effects	165
3. Respiratory Effects	166
4. Summary	167
B. The Effect of NH_4Cl on the Actions of Diamox	167
1. Pretreatment with NH_4Cl	167
(a) Acid-Base Excretion	169
(b) Excretion of Individual Ions	172
(c) Respiratory Effects	173
(d) Absorption and Excretion of Diamox in Metabolic Acidosis	173
2. Effect of NH_4Cl During Diamox Diuresis	174
C. Effect of Acidosis on the Role of Renal Carbonic Anhydrase	176
Discussion	
A. Renal	178
B. Respiratory	180
Conclusion	181

INTRODUCTION

The pattern of diminishing renal responses observed in certain schedules of treatment with Diamox® Acetazolamide (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide) suggested that urinary loss of HCO_3^- ("buffer base") and ensuing metabolic acidosis are the controlling factors in this alteration. Using the net balance of urinary $NH_4^+ + H^+ - HCO_3^-$ as a quantitative measure of acid-base balance, it was found for dosage schedules of Diamox ranging from 5 mg./kg./24 hours to 33 mg./kg./8 hours that the greater the acidosis, the less the renal effect (1).

[1] Presented in part at the Pharmacology Section of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., April 1954. Fed. Proc., 1954, **13:** 383.

[2] Formerly Instructor in Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine. Present address: Department of Pharmacology, University of Florida College of Medicine, Gainesville, Florida.

The present paper presents further evidence that renal resistance (i.e. lessening responses after successive doses) to Diamox results from the induction of metabolic acidosis; both base loss (following Diamox) or acid gain (following NH_4Cl) have been studied. Specifically four questions are asked:

1. What components of the metabolic acidosis are responsible for the altered renal and respiratory effects of Diamox?

2. What are the conditions and quantitative degree of acidosis required to produce renal resistance, and how are these related to the dose of Diamox?

3. In the setting of near maximal carbonic anhydrase inhibition, i.e. following large doses of Diamox: what fraction of filtered HCO_3^- is excreted, and to what extent is this figure altered in severe acidosis? In this alteration what is the role of Na^+ and K^+?

4. What metabolic or enzymatic alterations best explain renal resistance to carbonic anhydrase inhibitors in the face of base loss or acid gain?

Particularly with respect to the first two questions, conflicting data and comment have appeared in the literature (Table I). Some investigators have ascribed lowered renal effect of Diamox in acidosis to low plasma HCO_3^- (6, 7, 8), while our studies (1) pointed away from this relationship. It appeared likely that the varying results obtained with Diamox in acidotic dogs were due to different degrees of acidosis, as in the divergent results of Ref. 4 and Ref. 1. Little has appeared on the last two questions, except that in the normal dog (1) and man (8), Diamox causes excretion of about 20 per cent of filtered bicarbonate.[3] The data of Table I will be considered in relation to the present findings, in the discussion below.

METHODS AND MATERIALS

Animal techniques, chemical procedures, and mode of administration of Diamox were as previously described (1), with the following additions:

Experiments were done over a 16-month period, with many of the replicates done at 5- to 13-month intervals. Particular use was made of a well trained female beagle, P47, aged 4 years, which had been perineotomized 6 months prior to the onset of this work. Its weight varied in random fashion between 7.3–8.0 kg. throughout the experimental period. Its glomerular filtration rate (creatinine clearance) averaged 28 ml./min. Other dogs used were also trained female beagles. Procedures used in the cat experiments are described in Section C of the Experimental part. All animals were fasted on test days. Diamox and

[3] This figure indicates the maximal effect of Diamox under conditions where no infusions are used and initial urine flow is low. In comparing these results with somewhat greater effects (excretion of 23–39% of filtered HCO_3^-) in the dog (3) it appears that higher initial flow due to infusion of fluids may be responsible for the increased effect.

Quite a separate problem is posed by the work of Schwartz and Relman. They report excretion of up to 90% of filtered bicarbonate following massive intravenous doses of Diamox (5, 11). Those experiments are complicated by the simultaneous administration of about 50 meq. NaOH used to dissolve the Diamox, which results in severe transient *alkalosis*; the renal effect is apparently not related to carbonic anhydrase inhibition (24).

TABLE I

Published Observations on the Effects of Acidosis on the Renal Response to Diamox

Investigator	Ref.	Species	Amount and Type of Acid	Time of Acid Prior to Diamox	Authors' Conclusions
Berliner *et al.*	(3)	Dog	Not stated	Not stated	Diamox causes alkalinization of urine in acidotic dogs.
Berliner	(4)	Dog	1 meq./kg. HCl i.v.	2 hours	
Schwartz *et al.*	(5)	Dog	Not stated	Not stated	"Severely acidotic dogs responded less than those moderately acidotic." Leaf *et al.* (6) refer to (5), stating that "HCO_3^- diuresis that follows i.v. injection of drug has been found directly proportional to initial serum HCO_3^- concentration."
Maren *et al.*	(1)	Dog	None. Base *loss* following prior Diamox = 3–8 meq./kg.	Initial Diamox 24 hours before	"Depression of action of Diamox is roughly proportional to excess acid over base in the range 1–8 meq./kg. Plasma HCO_3^- concentration *per se* did not correlate well with either base depletion or diuretic activity."
Maren *et al.*	Footnote 1	Dog	Oral NH_4Cl, 5 meq./kg.	1 hour	
Madsen	(7)	Dog	NH_4Cl. Route and dose not given.	Not stated	"When the plasma bicarbonate concentration is low, the inhibitor has no effect."
Counihan *et al.*	(8)	Man	Oral NH_4Cl. Amount not given.	For 2 days prior to Diamox.	"There is . . . a linear relation between HCO_3^- excretion and initial plasma HCO_3^-, with no excretion of HCO_3^- occurring at plasma levels below 16 meq./l."
Kaye	(9)	Man	Oral NH_4Cl. 6 meq./kg.	12–24 hours before.	In normal man, NH_4Cl blocked the renal effect of Diamox. Plasma HCO_3^- was 15–16 meq./l.

Includes publications through June 1955, in order of their appearance. [Ref. 5 and Footnote 1 are abstracts.]

NH_4Cl were administered orally in gelatin capsules, except as noted. NH_4Cl was Merck Reagent Grade, Lot 31799.

EXPERIMENTAL

A. *Continuous Carbonic Anhydrase Inhibition*

Two experiments (Figs. 1 and 2) are shown in which continuous high plasma concentrations of Diamox were achieved by repeated intravenous injections in dogs. These concentrations (15–38 γ/ml.) are 5- to 10-fold greater than necessary for maximal renal effects (1). Figure 2 includes the recovery period after cessation of dosage.

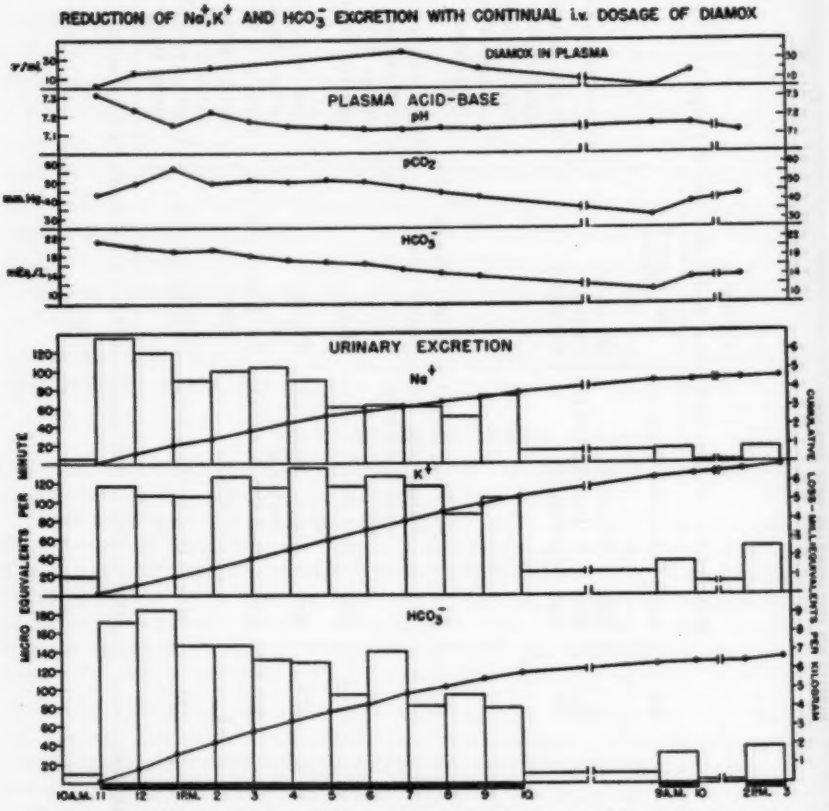


FIG. 1. Dog M84 ♀, 14.6 kg. Diamox, 10 mg./kg. intravenously, was given each hour beginning at 11 A.M., where noted by the black bar.

⌐¬ = μeq./min. excreted.

•——• = meq./kg., cumulative urinary loss.

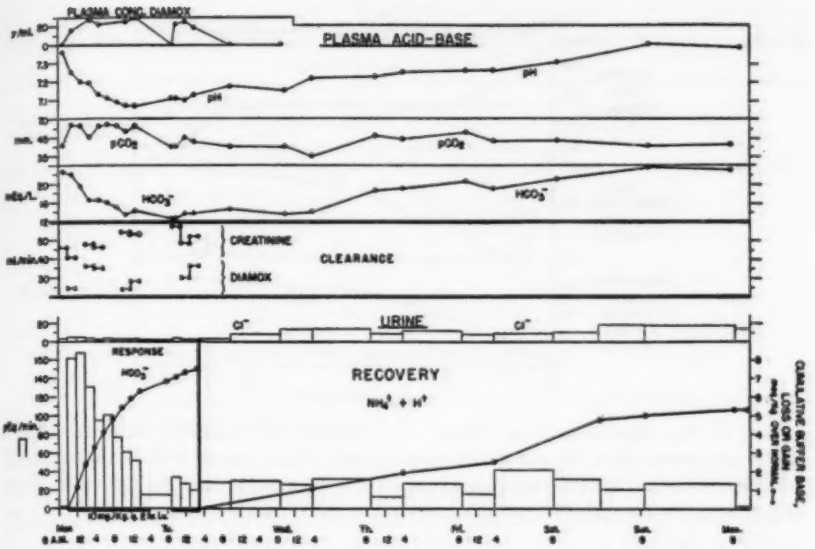


FIG. 2. Reduction of renal effect of Diamox with progressive loss of buffer base: recovery of acid-base balance. Dog P46, ♀, 17.7 kg. Diamox 10 mg./kg. intravenously was given every 2 hours, beginning at 10 A.M., where noted by the black bar.

□ = μeq./min. of HCO_3^- (response) or $NH_4^+ + H^+$ (recovery)

•——• = Cumulative urinary net base loss (response) or gain (recovery) over normal controls: $(NH_4^+ + H^+ - HCO_3^-)_{experimental} - (NH_4^+ + H^+ - HCO_3^-)_{control}$.

February 8, 1954 to February 15, 1954.

Figure 3 shows data on HCO_3^- filtration and reabsorption, derived from the experiment of Figure 2. The two experiments may be considered together under the headings of (*1*) Renal Excretion, (*2*) Metabolic (i.e. net renal acid-base) Effects, and (*3*) Respiratory Effects.

1. *Renal Excretion*

(a) *HCO_3^- Excretion.* In both experiments HCO_3^- excretion remained maximal for 4 and almost so for 6 hours, despite a decline of 5 meq./l. in plasma HCO_3^- in 5 hours. Thereafter, HCO_3^- excretion gradually declined, until following the final challenge of Figure 2 this value was 12 per cent of the original response. Figure 3 shows that about 80 per cent of the filtered HCO_3^- (middle curve) was reabsorbed in the first 4 hours of dosage; thereafter and with progressive loss of body HCO_3^- (lowest curve), this figure rose to 97 per cent. The rate of reabsorption of HCO_3^- in this situation is clearly not a constant fraction of that filtered.

The two lower curves of Figure 3 suggest that the decline in effect is closely

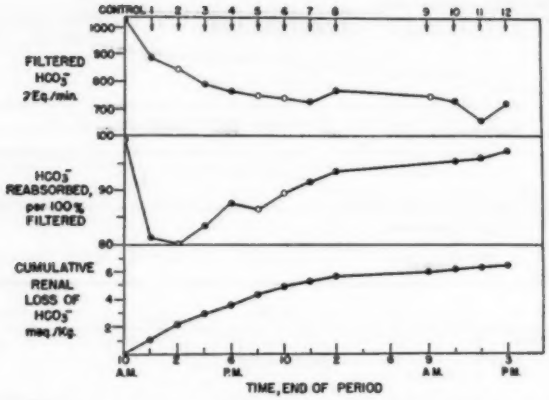


FIG. 3. The relation between HCO_3^- reabsorption and metabolic acidosis. Plotted points and arrows show the *end* of treatment periods. First dose at 10 A.M. 10 mg./kg./2 hours intravenously for 9 doses, then a lapse of 7 hours and the schedule continued. Open circles based on interpolated creatinine clearances. Same experiment as Figure 2.

correlated with cumulative renal loss of HCO_3^-; the top curve shows that after the third period the amount of filtered bicarbonate declined but slightly[4] during radical changes in HCO_3^- reabsorption. It has been found previously that during continued oral dosage with Diamox, plasma HCO_3^- concentration, after an initial fall, stays constant while HCO_3^- excretion declines gradually (Ref. 1: Fig. 11). Table II shows a model of this phenomenon, which has invariably been observed when high plasma concentrations of Diamox have been maintained around the clock. There appears to be a rapid exchange of HCO_3^- between plasma and other compartments. Since Diamox may elicit excretion of HCO_3^- with no change in plasma HCO_3^- concentration, it is clear that plasma HCO_3^- cannot be a measure of total body HCO_3^-.

Figures 1 and 2 indicate that in metabolic acidosis due to loss of HCO_3^-, the renal effect of Diamox is reduced. This reduction, although gradual, becomes significant when about 4–7 meq./kg. of HCO_3^- has been lost, a range which agrees with previous studies with oral Diamox (1), and with the experiments to be described (Section B, below) on induced acidosis with 5 meq./kg. of NH_4Cl.

(b) *Na^+ Excretion.* Figure 1 shows that following hourly injections of Dia-

[4] This results from the fact that from the 5th to the 13th hour (3 P.M. to 11 P.M.) the moderate decline in plasma HCO_3^- concentration was matched by an increase in creatinine clearance (Fig. 2). Such an increase concomitant with the development of acidosis has been observed previously, both in the first day (1) and in long-term treatment (2). A possible explanation is an increased cardiac output which has been observed in acidotic dogs (Poppell, J. W. *et al.*, Clinical Res. Proc., 1955, **3**: 104).

TABLE II

A Model of Urinary Electrolyte Patterns at Various Degrees of HCO_3^- Depletion Following Continuous Diamox Treatment in the Dog

Time of Exposure to Diamox (Hrs.)[b]	Midpoint Plasma		Urinary Excretion μeq./min.			Cumulative Loss of HCO_3^- (meq./kg.)[a]	% Excreted/ Filtered HCO_3^-
	HCO_3^- (meq./l.)	pCO_2 (mm. Hg)	HCO_3^-	Na^+	K^+		
0–4	20	45	160	110	110	0	20
4–8	16	55	110	60	100	2.4	17
8–16	12	48	50	30	50	4.1	10
16–24	12	40	16	10	30	5.5	3
Withdrawal[b]							
24–32	12	30	4	3	10	6.1	1

Assume constant filtration rate of 40 ml./min. and weight of 16 kg.

[a] At start of each period.

[b] Assume that plasma concentration of Diamox is above 5 γ/ml. from 0–24 hours, and declines to 1–2 γ/ml. at midpoint of withdrawal period.

mox, Na^+ excretion declines in a manner quantitatively like that of HCO_3^-. This parallelism has also been observed in long-term experiments with Diamox (1), as well as in acute experiments with NH_4Cl and Diamox (Section B, below). The cumulative sodium loss was 4.6 meq./kg. for the 28-hour period, or about 9 per cent of total body sodium. About the same deficit is observed in long-term experiments, supporting the conclusion that no net loss of sodium occurs after day 1 in the normal dog (1).

(c) *K^+ Excretion.* Figure 1 shows that K^+ excretion continues elevated longer than does Na^+. As a result of this prolonged action, the cumulative loss was greater than that of Na^+, being 6.8 meq./kg., or 17 per cent of total body K^+. This agrees with earlier studies of long-term treatment with oral Diamox in which progressive net potassium loss did not extend past the first or second day, and where depletion at the end of 16 months was estimated at 15 per cent of body K^+ (1).

When the effect of Diamox is partially blocked by metabolic acidosis, the K^+/Na^+ ratio in urine generally rises, often to a value above 2. This is seen in the final challenge of Figure 1. The ratio in the diet and in control urines is 1.5 (1), and the ratio in the initial periods after Diamox is approximately 1. Table II shows a model of the changing pattern of Na^+ and K^+ excretion with the development of acidosis and renal resistance to Diamox.

2. *Metabolic Effects*

Metabolic acidosis is defined as loss or gain of acid. Figure 1 shows cumulative renal loss of HCO_3^- as a measure of this change. Figure 2 shows the more precise measurement of net base loss or gain, i.e. the difference between urinary base economy on experimental and on control days: ($NH_4^+ + H^+ -$

$HCO_3^-)_{experimental} - (NH_4^+ + H^+ - HCO_3^-)_{control}$ for both the day of Diamox treatment and recovery days. Control values are taken as +24 meq./day (1) or +0.06 meq./kg./hour.[5] Since the control values are relatively small and excretion of $NH_4^+ + H^+$ is negligible in the first day of Diamox, the renal loss of HCO_3^- (Fig. 1) is a fair approximation of the net base deficit (Fig. 2).

Cumulative excretion of HCO_3^- (Fig. 1) or net base (Fig. 2) totalled 6.6 and 7.4 meq./kg., respectively. This is about the maximum loss that can be elicited by Diamox (1). The lowest value for plasma HCO_3^- concentration was 11.0 meq./l., corresponding to blood pH of 7.16 (Fig. 1, 9 A.M., day 2). Diamox does not appear able to drive plasma bicarbonate below 11 meq./l.; but at that approximate concentration, depending on total body stores of bicarbonate, the renal effect can be almost absent (day 2 in Figs. 1 and 2), or partial (8–16 hours in Figs. 1 and 2), or normal (in respiratory "alkalosis"; see Ref. 1). The greater the depletion of *body* bicarbonate, the less the renal effect.

When Diamox was discontinued (Fig. 2, 2 P.M. Tuesday), the usual effects in the recovery of acid-base balance were observed. Supernormal excretion of NH_4^+ and H^+ were apparent for 5 days; during that time plasma bicarbonate and pH climbed gradually back to normal. Of the 7.4 meq./kg. net loss of base resulting from Diamox treatment, it was possible to account for 5.3 meq./kg. in the recovery period, in terms of excess $NH_4^+ + H^+$ output or base gain. It is of interest that the recovery period was about four times as long as the depletion period; this has also been observed in other schedules (1). Base depletion, i.e. acidosis, of the order of 7 meq./kg. is not a stimulus for maximal urinary acidification rates; nor is 5 meq./kg. of NH_4Cl (see below). At this approximate level of acidosis (which is the greatest that appears possible following Diamox), urinary excretion of $NH_4^+ + H^+$ does not exceed 50 μeq./min. However, after 12 meq./kg. of ammonium chloride, urinary excretion of $NH_4^+ + H^+$ is variable but may reach a maximum of 140 μeq./min. (see below). Thus the recovery from acidosis induced by Diamox is characterized by a moderate rate but prolonged time of extra urinary acidification.

In the experiment of Figure 2, chloride excretion was low during administration of Diamox and approximately normal during recovery, in agreement with previous data (1).

3. *Respiratory Effects*

In the preceding discussion of the metabolic acidosis following continuous intravenous Diamox, it was noted that there was a fall of plasma bicarbonate and pH. Both Figures 1 and 2 show a rise in pCO_2 during the first 12 hours of drug administration. A rise in pCO_2 is opposite to the usual effect in metabolic acidosis following ammonium chloride, and almost certainly indicates inter-

[5] Diurnal periodicity of ion excretion in control periods, which is relatively small in fasting dogs (1), is neglected in these figures.

ference with elimination of CO_2 from the blood. Such an elevation in pCO_2 is usually observed following intravenous Diamox, but may not be seen following oral Diamox (1). Elevation of pCO_2 is more clearly demonstrated when renal loss of HCO_3^- (which tends to lower pCO_2) is blocked; in Figures 1 and 2 this is evident following the dose at 9 A.M. on day 2. The rise in pCO_2, now unopposed by renal loss of HCO_3^-, leads to elevation of plasma HCO_3^-, the familiar pathway in the production of compensated respiratory acidosis.

The acidosis produced by Diamox is both respiratory *and* metabolic in character. When Diamox concentration in plasma declines to zero (Figs. 1 and 2) but before the base deficit can be repaired, pCO_2 drops, disclosing a compensated metabolic acidosis. In this situation, pCO_2 is almost as low as that following NH_4Cl. This phenomenon supports the view that Diamox was responsible for elevation of pCO_2, but raises the difficult question as to why elevation of *plasma* concentration of drug coincides with elevation of pCO_2. One might expect rather that *erythrocyte* concentration of drug would affect pCO_2, since carbonic anhydrase is in red cells. Although erythrocyte concentrations of Diamox were not measured in these two experiments, it has been established beyond question that Diamox concentration in red cells persists even after a single dose, virtually unchanged for 24 hours (2). The drop in pCO_2 observed at 9 A.M. on day 2 in both experiments thus coincides with a drop in plasma Diamox, and not with a drop in red cell Diamox or with any substantial change in acid-base balance.

4. *Summary*

Figure 2 indicates that there are no striking abnormalities in plasma concentrations or clearances of Diamox following intravenous administration every 2 hours. This agrees with previous work on continued oral administration (2). The clearance of both creatinine and Diamox (Fig. 2) appears to rise somewhat under conditions of continued administration. Changes in the renal effects of Diamox with continuous dosage do not appear due to alteration in the pharmacological disposition of drug.

Table II summarizes in idealized form the findings of the present section and related experiments in (1). It is evident that resistance to renal effects of Diamox increases as base depletion *and* time of exposure to drug increase. These factors have not been separated. In the following section, the time factor has been held more or less constant, and the degree of acidosis varied.

B. *The Effect of NH_4Cl on the Actions of Diamox*

1. *Pretreatment with NH_4Cl*

Table III shows an experiment in which a large total dose of NH_4Cl completely blocked the renal effect of Diamox at 10 mg./kg., a dose that had never before (1) failed to elicit near maximal urinary excretion of HCO_3^-. In the

TABLE III

Response to 20-Hour NH_4Cl Acidosis—Diamox 10 mg./kg. Orally at 0 Time

Hours After Dose	Plasma			Urine									
						μeq./min.						Diamox	
	pH	HCO_3^- (meq./l.)	pCO_2 (mm. Hg)	Flow (ml./min.)	pH	HCO_3^-	Cl^-	T.A.	NH_4^+	Na^+	K^+	mg. per Sample	% Dose Cum.
−2					5.2								
0	7.10	9.7	31	0.34	5.3	<1	73	16	100	14	30		
0–3				0.97	5.1	<1	208	25	97	89	98	13	10
3–6	7.11	12.6	42	0.79	5.1	<1	114	22	71	42	57	32	33
	6 hour totals.....			318 ml.	5.1	meq...	57	9	30	24	28	45 mg.	33%

On the preceding day, the animal was fed at 1 P.M. Then it was given 1 g. of NH_4Cl every 20 minutes to a total of 6 g. (8 meq./kg.). At −2 hours the next morning it was fed, then NH_4Cl was resumed 1 g. every 15 minutes to a total of 4 g. (5.5 meq./kg.). Diamox was given at 0 time. Water intake 0–6 hours, 410 ml. In this and the following Table, plasma values refer to end of period.

The data for urinary recovery of Diamox, compared to previous studies (2), show that absorption and/or excretion of drug is slightly but not seriously reduced by this treatment.

Dog M84, ♀, 14.3 kg. July 29, 1953

absence of the renal effect, Diamox caused a rise in pCO_2. Table IV shows the effect of a similar dose of NH_4Cl on the renal effect of Diamox at 100 mg./kg. given intravenously. In this case HCO_3^- excretion was not entirely blocked, but reduced to about ¼ of the rate found following Diamox alone. Diamox after NH_4Cl again caused a pronounced elevation in pCO_2. Further experiments, which form the substance of this section, were carried out in an attempt to obtain quantitative data on these phenomena.

Table V summarizes 29 experiments in a single dog, in which standardized procedures of NH_4Cl and subsequent Diamox administration were carried out, together with appropriate controls. Oral doses of Diamox were used to avoid artefacts, such as lowering of glomerular filtration rate and effects due to large amounts of sodium, that occur when the intravenous preparation is used. Absorption is sufficiently rapid so that near peak plasma concentrations are achieved by the end of the first hour and the onset of renal effect within 30 minutes (2). The NH_4Cl ranged from the usual clinical dose (2 meq./kg.) to an amount (12 meq./kg.) that elicited a more severe acidosis than was possible by the use of Diamox alone. The lower dose of Diamox has been shown to give virtually maximal HCO_3^- excretion both in dog (1) and man (8); plasma concentrations in the dog were 2–5 γ/ml. The higher dose was used to approach 100 per cent inhibition of renal enzyme with some certainty; the plasma concentrations of Diamox 3 hours after this dose were variable but averaged about 10 times the values which corresponded to that necessary for maximal renal effects. Table V indicates that 10 mg./kg. gives nearly complete inhibition of the enzyme, since there is considerable overlap between these effects and those following 100 mg./kg.

TABLE IV

Effect of Severe NH_4Cl Acidosis on Massive Intravenous Diamox

Time (hrs.)	Plasma			Urine						Diamox	
	pH	HCO_3^- (meq./L)	pCO_2 (mm. Hg)	Flow (ml./min.)	pH	μeq./min.				mg.	% Dose cumul.
						HCO_3^-	Cl^-	Na^+	K^+		
A											
At 0 Time: Diamox 100 mg./kg. i.v.: No NH_4Cl											
−1–0	7.30	21.8	45	0.08	5.8	0	1	4	7		
0–2				1.02	8.0	193	11	134	145	504	40
2–4	7.17	17.1	48	0.54	7.8	76	4	70	58	121	50
B											
NH_4Cl: 1 g. every hour x 6 on previous day; 1 g. every 15 min. x 8 from −3 to −1 hours. Total acid* = 17 meq./kg. Diamox 100 mg./kg. i.v. at 0 time.											
−3 to −2	7.31	11.9	23	0.23	6.8	11	34	12	3		
−2 to −0	7.07	9.8	29	0.42	5.0	0	92	36	26		
0–2	6.93	10.7	46	1.66	7.4	66	184	171	185	204	13
2–4	6.94	10.9	46	1.56	6.7	20	145	103	174	119	21

Dog M79, ♀. A. Wt.: 13 kg. Sept. 2, 1954 B. Wt.: 15 kg. July 31, 1953

* Since almost half of this was given on the previous day, it does not represent the acid load at the time of Diamox injection. This load was at least 10 meq./kg., the amount given within 3 hours of injection time.

Table VA is included to show urinary composition at the time Diamox was given (zero time) in these experiments, i.e. following acute loading with NH_4Cl. The urine does not markedly reflect these loads, except at 12 meq./kg., which was given over a longer period of time (210 minutes) than the lower doses.

The data of Table V are considered with respect to (a) Acid-Base Excretion, (b) Excretion of Individual Ions, (c) Respiratory Effects, and (d) Absorption and Excretion of Diamox.

(a) *Acid-Base Excretion.* As indicated above, the renal effect of Diamox may be gauged by the difference between urinary base economy, i.e. excretion of $NH_4^+ + H^+ - HCO_3^-$, in control and in treatment periods. Figure 4 treats the data of Table V in this fashion: the base economy for each NH_4Cl load is subtracted from the base economy for the same NH_4Cl load + Diamox. The results show the net loss of base due to Diamox, at NH_4Cl loads from 0–12 meq./kg. Regarding the experiments with Diamox at 100 mg./kg. as representing complete inhibition of renal carbonic anhydrase, it is evident that excretion of base is unaffected by 2 meq./kg. NH_4Cl but is progressively diminished at 5 and 12 meq./kg. NH_4Cl.

It is of interest that 2 meq./kg. of base depletion following Diamox does not block its renal effect, but in the range 3–8 meq./kg. such blocking increases

TABLE V

Three-Hour Excretion of Electrolytes After Successive Oral Doses of NH_4Cl and Diamox

NH_4Cl[a] (meq./kg.)	Diamox[b] (mg./kg.)	Plasma at 0 Time		Plasma at 3 hrs.		Urinary Excretion 0–3 Hrs. after Diamox								Diamox at 3 hrs.	
		pH	HCO_3^- (meq./L)	pH	HCO_3^- (meq./L)	Flow (ml./min.)	pH	μeq./min.[c]						Plasma (γ/ml.)	Urine % Dose
								HCO_3^-	Cl^-	NH_4^+	H^+	Na^+	K^+		
0	0	7.24	23	7.33	22	.07	7.7	6	—	—	—	3	11		
0	0	—	—	7.33	23	.23	6.8	4	—	4	1	7	10		
0	0	—	—	—	—	.10	6.7	2	2	3	1	7	16		
2	0	7.33	19	7.34	17	.09	7.8	7	1	.3	0	12	13		
2	0	7.25	18	7.31	16	.12	7.1	5	16	2	0	13	28		
5	0	7.23	13	7.27	13	.24	5.2	0	55	9	5	15	48		
5	0	7.28	14	7.29	13	.10	5.5	0	23	4	2	5	16		
12	0	7.02	6	7.07	6	.35	5.2	0	119	27	15	27	90		
12	0	7.21	11	7.22	11	.39	5.5	0	16	5	8	2	18		
0	10	7.35	22	7.21	—	.17	8.0	42	2	0	0	37	23		
0	10	7.35	23	7.25	21	.20	8.1	73	2	0	0	52	39		
0	10	7.31	21	7.22	20	.19	8.1	50	—	0	0	38	28		
0	10	7.28	20	7.24	19	.25	8.0	66	—	0	0	54	32		
2	10	7.31	19	7.32	17	.26	8.1	41	19	1	0	47	49		
2	10	7.30	18	7.23	17	.10	7.7	11	9	0	0	16	17		
5	10	7.15	17	7.13	14	.33	7.4	5	82	6	0	3	34		
5	10	7.23	16	7.23	14	.29	7.6	13	50	4	0	32	28		
12	10	7.09	11	7.05	12	.21	5.7	0	83	15	6	5	76		
12	10	7.18	10	7.14	11	.65	7.0	13	33	1	1	10	87	8	31
0	100	7.35	19	7.16	16	.48	8.0	84	1	1	0	95	67	39	16
0	100	7.34	21	7.20	16	.29	8.1	61	7	0	0	60	39	17	5
0	100	—	—	—	—	.34	8.0	76	—	—	—	—	—	12	5
0	100	—	—	—	—	.52	8.0	89	—	—	—	—	—	57	11
2	100	7.29	18	7.17	16	.48	7.9	85	16	1	0	81	85	36	14
2	100	7.32	17	7.11	16	.36	7.9	81	9	1	0	65	44	49	10
5	100	7.25	16	7.15	14	.33	7.9	39	62	—	0	40	74		5
5	100	7.19	12	7.09	13	.49	7.6	47	50	0	0	39	107	43	13
12	100	7.12	9	7.12	11	.27	7.2	8	41	4	0	13	35	18	3
12	100	7.12	9	7.12	11	.25	7.0	10	72	6	12	23	73	13	3

[a] By capsule. For 2 meq./kg., 0.4 g. at −60 and −40 min.; 5 meq./kg., 1.0 g. at −60 and −40 min.; 12 meq./kg., 1.0 g. every 20 min. × 5, starting at −210 min.

[b] By capsule at 0 time.

[c] Entry of 0 is made for values below 0.5 μeq./min.

Dog P47, ♀; wt. 7.3–8.0 kg.
October 1953–December 1954.

TABLE VA

The Effect of Oral Acute NH_4Cl on Urinary Composition (Supplement to Table V)

NH_4Cl (meq./kg.)	No. of Det'ns	Urinary Composition after NH_4Cl, Prior to Diamox							
		Flow (ml./min.)	pH	HCO_3^-	Cl^-	NH_4^+	T.A.	Na^+	K^+
				microequivalents/minute					
0	6	0.13	7.7	9	4	<1	0	8	17
2	4	0.14	6.3	1	8	7	2	3	12
5	4	0.12	6.1	<1	10	7	1	5	10
12	4	0.38	5.6	<1	91	24	6	19	50

Average figures for the pre-Diamox periods in the experiments of Table V. 0, 2, and 5 meq./kg. of NH_4Cl in 60-minute periods; 12 meq./kg. in 210 minutes.

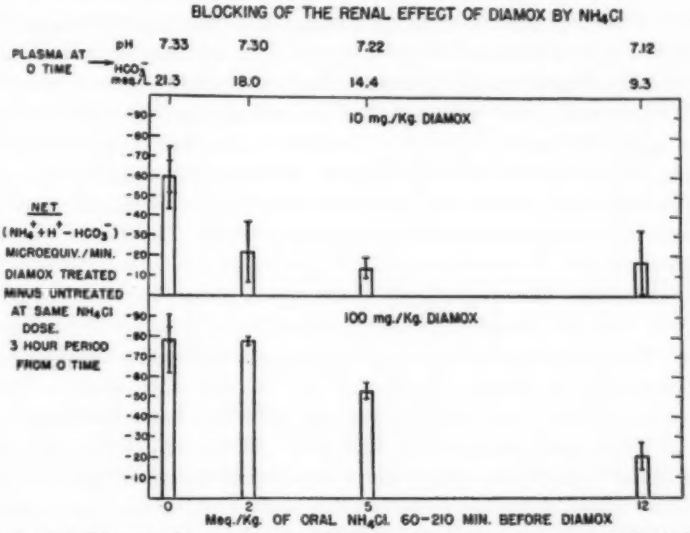

BLOCKING OF THE RENAL EFFECT OF DIAMOX BY NH4Cl
PLASMA AT O TIME
pH 7.33 7.30 7.22 7.12
HCO3 meq/L 21.3 18.0 14.4 9.3
10 mg./Kg. DIAMOX
100 mg./Kg. DIAMOX
NET (NH4+ + H+ − HCO3−) MICROEQUIV./MIN. DIAMOX TREATED MINUS UNTREATED AT SAME NH4Cl DOSE. 3 HOUR PERIOD FROM O TIME
Meq./Kg. OF ORAL NH4Cl. 60–210 MIN. BEFORE DIAMOX

FIG. 4. Oral Diamox given at 0 time. Heights of bars are averages of the individual points plotted on the vertical lines. Plasma values are averages. Individual data in Table V. All experiments in Dog P47, ♀; wt. 7.6–8.0 kg.

in proportion to the acidosis (1) just as seen in Figure 4 for NH_4Cl loads. The data of Tables V and VA also suggest an answer to the question as to why the acidosis in long-term Diamox treatment is not overcome by the dog, since it is evident that some H^+ and NH_4^+ can be excreted in such animals (1). Tables V and VA show that 2–5 meq./kg. of acid is insufficient stimulus for *large* urinary output of H^+ and NH_4^+; in the long-term Diamox experiments, base loss rarely exceeded 5 meq./kg. Thus the dog appears relatively indifferent either to addition of acid or loss of base below about 12 meq./kg., in terms of stimulation of renal compensatory mechanisms. Figure 4 shows that doses of acid below 12 meq./kg. do not greatly lower the effects of high doses of Diamox.

Following 12 meq./kg. of NH_4Cl, the net renal effect of oral Diamox at either 10 or 100 mg./kg. is reduced to approximately ¼ that of Diamox alone. It appears that when this dog is in normal acid base balance, carbonic anhydrase can facilitate the net conservation of 60–80 μeq./min. of base; this is the quantity lost when the enzyme appears completely inhibited. The remainder of base conservation is accomplished by a different (non-enzymatic or another enzymatic) process. In severe acidosis, the net conservation of only 15–20 μeq./min. of base is under control of the enzyme. Again, the remainder is handled by a different process. In summary, in severe metabolic acidosis, carbonic anhydrase has less of a role in the conservation of bicarbonate than it does in normal acid-base balance.

(b) *Excretion of Individual Ions.* HCO_3^-: The data of Table V permit an estimate of bicarbonate excretion, in terms of the approximate amount filtered at various levels of Diamox dosage and of NH_4Cl acidosis. Plasma bicarbonate concentration did not change greatly in the 3-hour interval; the average of the 0- and 3-hour values was used as a basis for calculations. Neither oral Diamox (2) nor high acid loads (10) depress creatinine clearance (assumed equivalent to glomerular filtration) in the dog. In separate experiments, the average creatinine clearance of this dog (P47) was found to be 28 ml./min.; this figure was used to calculate the rate of bicarbonate filtered per minute throughout the experiment.

For the entire 3-hour period, oral Diamox at 100 mg./kg. elicits excretion of 12–18 per cent of filtered bicarbonate when there is no or 2 mg./kg. prior NH_4Cl.[6] This figure agrees with data of Figure 3, and again suggests that it is only this moiety of filtered bicarbonate that is reabsorbed under influence of carbonic anhydrase. Five and 12 meq./kg. of NH_4Cl respectively reduce this figure to 10 per cent and to 3 per cent. With 10 mg./kg. (Tables III and V), high doses of NH_4Cl can completely eliminate HCO_3^- excretion. These data on HCO_3^- excretion/filtration rates agree with those of *net* base loss in the previous section, i.e. metabolic acidosis reduces the maximal effects of Diamox, measured in either fashion, to about ⅕ of the normal value.

Cl^-: The chloruresis elicited by NH_4Cl was variable in degree and did not appear to be affected by the subsequent administration of Diamox.

Na^+: The Cl^- excreted in response to NH_4Cl alone was largely matched by K^+; there was little Na^+ effect. When Diamox was given subsequent to NH_4Cl, Na^+ output appeared to depend on HCO_3^- excretion; increasing amounts of NH_4Cl, at each of the two dose levels of Diamox, progressively diminished Na^+ excretion.

[6] This range of 12–18% is slightly low because it includes a small period of time (~30 minutes) during which drug is being absorbed and urinary excretion of HCO_3^- is relatively low. If calculations are based on the 90- to 180-minute period after dose, the range of excreted/filtered bicarbonate following Diamox alone at 100 mg./kg. or with 2 meq./kg. NH_4Cl is 11–24%. This is reduced to 4–6% following 12 meq. NH_4Cl and 100 mg./kg. Diamox.

K^+: The chloride excretion following NH_4Cl alone was usually matched by K^+. When Diamox was given, K^+ appeared (with Na^+) to match HCO_3^- if there was a typical effect, or to match chloride if prior NH_4Cl elicited a chloruresis.

H^+: Excretion of H^+ and NH_4^+ increases in proportion to the acid load either with or without Diamox. Due to the relatively short time interval following NH_4Cl in the experiments of Table V, $H^+ + NH_4^+$ output never reached high levels even in the absence of Diamox. Under these conditions, Diamox and NH_4Cl usually caused lower output of $H^+ + NH_4^+$ than NH_4Cl alone. The summation of these effects with HCO_3^- output is given in Figure 4. The highest rate of NH_4^+ and H^+ excretion was observed in the longer experiment of Table III; in this case Diamox (10 mg./kg. orally) did not reduce this rate.

(c) *Respiratory Effects.* Plasma pCO_2 may be calculated from data of Table V. NH_4Cl alone invariably lowers pCO_2; this is the well-known pathway of respiratory compensation for metabolic acidosis. The varying effects of Diamox alone on pCO_2 have been discussed (1) and are noted here in relation to Figures 1 and 2 (Section 3, above).

The variations in pCO_2 obtained with initial doses of Diamox are dispelled when the renal effect is abolished or diminished by previously administered NH_4Cl (Tables III–V). In this setting, Diamox invariably causes a rise in pCO_2. This rise is often accompanied by a rise in plasma HCO_3^-. It seems clear that when Diamox does not elicit a loss of base (which tends to lower pCO_2), the inhibition of blood carbonic anhydrase results in an elevation of pCO_2 (respiratory acidosis), now unopposed by the "metabolic" drive in the opposite direction.

(d) *Absorption and Excretion of Diamox in Metabolic Acidosis.* It is essential to ask if the alteration of activity of Diamox in metabolic acidosis is due to altered behavior of the drug in the new physiological environment. Such does not appear to be the case in acidosis due to Diamox itself (1, 2; present Figs. 1–2). In the more severe acidosis due to 12 meq./kg. of NH_4Cl, there are some alterations in the pharmacology of Diamox,[7] but these do not appear to influence the present study. The plasma concentrations of Diamox following 100 mg./kg. alone, are similar in two instances to those following 12 meq./kg.

[7] Table V shows that absorption of Diamox at 100 mg./kg. is erratic, either alone (see also Ref. 2) or following NH_4Cl. Excretion of this dose of Diamox appears to be depressed by 12 meq./kg. of NH_4Cl. Table IV also shows, 0–2 hours after intravenous dosage, a lower rate of Diamox excretion in the acidotic compared with the normal dog. 2–4 hours after dosage, there was no difference. This is consistent with the thesis that small amounts of Diamox may be reabsorbed regardless of the pH of final urine, but larger amounts, which contribute considerably to the electrolyte load in urine (i.e. 19 μmoles/min. at 0–2 hours in part A of Table IV), are affected by urinary acidity. As with HCO_3^- and other weak acids (*cf.* barbital, Ref. 12), renal reabsorption of Diamox (pKa = 7.4) is increased as urinary pH declines, presumably due to the increased fraction of the unionized (acid) over the ionic species.

NH_4Cl and Diamox at 100 mg./kg. (Table V). Despite the similar plasma concentrations (all four in the range 12–18 γ/ml.), renal effects were strikingly different. We have shown previously that for an initial dose of Diamox alone the renal effect depends on maintenance of a plasma concentration of over 2 γ/ml.; in all of the experiments reported here this value is considerably exceeded. The use of 100 mg./kg. ensures higher plasma and urine concentrations than are obtained in non-acidotic dogs at 5–10 mg./kg. where there is nearly complete inhibition of renal enzyme (Fig. 4). We conclude that the effects of NH_4Cl on renal carbonic anhydrase inhibition are not due to alteration in the absorption or excretion of Diamox.

2. *Effect of NH_4Cl During Diamox Diuresis*

Previous experiments showed the effect of *prior* Diamox or NH_4Cl acidosis on the renal action of Diamox. Figure 5 shows the effect on Diamox diuresis, of NH_4Cl given *after* onset of the Diamox effect (solid line). It is evident that NH_4Cl started 40 minutes after injection of Diamox completely abolished the effect of Diamox on HCO_3^- excretion within 140 minutes, or 3 hours after injection of inhibitor. Without NH_4Cl, this dose of Diamox (1) will uniformly maintain an alkaline urine for at least 6 hours (dotted line in Fig. 5 shows this effect, with at least 60 μeq. of HCO_3^- excreted per minute for 4½ hours). The NH_4Cl, at 3 hours, elicited acid excretion of 22 μeq./min. in the presence of Diamox, a rate 3–5 times over that in normal untreated dogs (compare Table V).

The decline in renal activity of Diamox induced by NH_4Cl was accompanied by a large rise in chloride excretion. The following are changes in excretion rates (μeq./min.) between the onset (period between 100–160 minutes) and height (period between 180–200 minutes) of chloruresis. *Anions:* Cl^-, +79; $PO_4^=$, 0; HCO_3^-, −16; net anion increase, 63. *Cations:* H^+ and NH_4^+, +19; K^+, +33; Na^+, −10; net cation increase, 42. The chloride increase is partially matched by H^+ and K^+, sodium being unaffected; this agrees with previous data for 5 and 12 meq. of NH_4Cl, both with and without Diamox (Table V). We have previously noted the accompaniment of urinary K^+ and Cl^- in the long-term acidosis induced by Diamox (1).

The reversal of Diamox effect on urinary HCO_3^-, by ammonium chloride, in Figure 5 (solid line) was followed by re-challenge with Diamox 200 minutes after the first injection and during the severe acidosis. (Without NH_4Cl, a second or even third injection within this time would result in a prolonged renal effect similar to the first injection, as seen in Figs. 1 and 2.) The second injection of Diamox resulted here in a moderate elevation of urinary bicarbonate for the first period, followed by a fairly sharp decline. Table VI compares the immediate changes in excretion which followed Diamox at zero time with those that followed Diamox at 200 minutes in the dog now rendered acidotic.

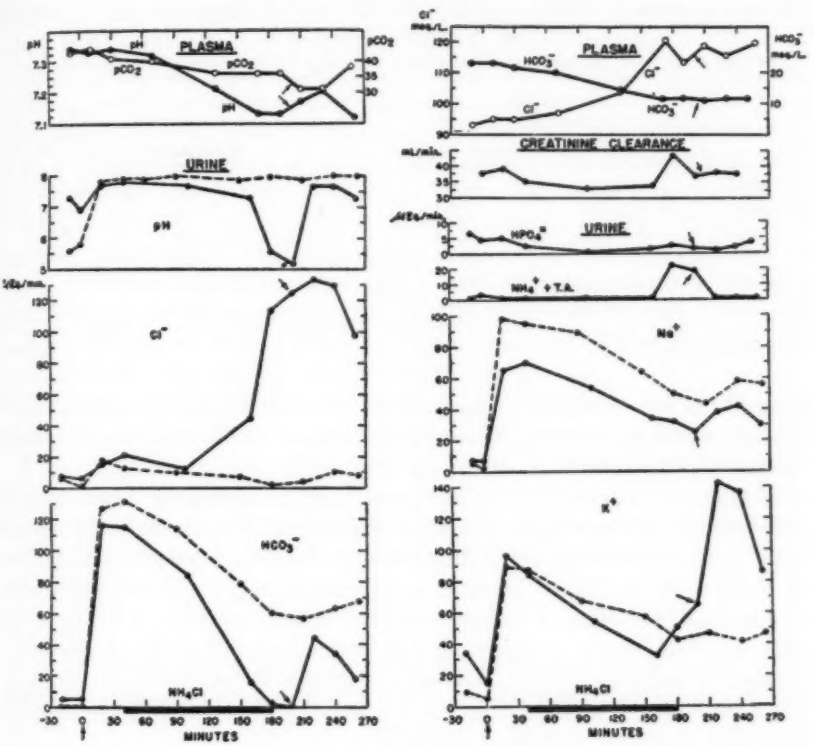


FIG. 5.

——— Dog P43, ♀, 11.5 kg. Urine and Plasma. Diamox 5 mg./kg. intravenously at arrows, 0 time and 200 min.; oral NH_4Cl (black bar) 0.7 g. every 20 min. x 7 doses = 8 meq./kg.; at −60 min., 300 mg./kg. subcutaneously of creatinine and 20 ml./kg. orally of water.

– – – – – Dog P46, ♀, 17.3 kg. "Control" urinary output following Diamox 5 mg./kg. at first arrow (0 time) only. Both dogs fasted on test day.

March 16, 1954 and April 6, 1954.

Using the expression of change in $NH_4^+ + H^+ - HCO_3^-$ as an index of Diamox activity, we find that after the initial challenge, this value was −107; after challenge at 200 minutes, it was −52. The cation effect in the acidotic phase was transient and entirely limited to potassium; the K^+ increment (65 μeq./min.) is matched by increase of HCO_3^- (32 μeq./min.) and decline in $H^+ + NH_4^+$ (20 μeq./min.).

The respiratory effect of Diamox in the experiment of Figure 5 is evidenced by the relatively high (36 mm. Hg, from 130 to 200 minutes) pCO_2 in the

TABLE VI

The Effect of Intravenous Diamox (5 mg./kg.) Alone (A), and 200 Minutes Later After Induction of Severe Metabolic Acidosis (B)

Average changes in rate (μeq./min.) between 2 periods prior and 3 following injection.

	Anions			Cations	
	A. Normal 0 time	B. Acidotic 200 min.		A. Normal 0 time	B. Acidotic 200 min.
Cl^-	+11	+1	H^+	0	−12
HCO_3^-	+105	+32	NH_4^+	−2	−8
$PO_4^=$	−3	+1	K^+	+59	+65
			Na^+	+59	+8
Sum.....	+113	+34		+116	+43

Experiment of Figure 5.

presence of severe NH_4Cl acidosis. Were there no Diamox present, NH_4Cl acidosis would be expected to lower the pCO_2 to 22–29 mm. Hg, the range calculated from Table V.

In summary, the data of Figure 5 show that: (a) ammonium chloride given *after* Diamox, and during its peak renal effect completely destroys that effect; (b) a small intravenous dose of Diamox in the acidotic dog has half the effect on $NH_4^+ + H^+ - HCO_3^-$ excretion as the same dose given 3 hours previously to the same dog when it was in normal acid-base balance. In acidosis the Diamox effect is more transient than in the normal, and is characterized by absence of sodium excretion, potassium matching the alteration in $NH_4^+ + H^+ - HCO_3^-$; (c) pCO_2 is higher in the presence of Diamox than in similar metabolic situations without inhibitor.

C. *Effect of Acidosis on the Role of Renal Carbonic Anhydrase*

The physiological basis for the diminished renal effect of Diamox in metabolic acidosis is considered in the present section. The foregoing data show that the change is not a direct function of filtered or plasma HCO_3^- (Fig. 3; Table V), or due to marked changes in urinary composition (Table VA), or due to altered pharmacological disposition of inhibitor (Fig. 2; Tables III–V). Two other possibilities may be briefly explored:

1. In metabolic acidosis, renal carbonic anhydrase activity might temporarily disappear, or increase, or change in some fashion so that it is no longer susceptible to inhibition by Diamox.[8]

The temporary nature of any possible alteration is indicated by the rapid reversibility of effects following administration of Diamox (1).

[8] In the elasmobranch kidney, which does not respond to Diamox, there is virtually no enzyme; urinary pH is fixed at 5.8 (13).

Any change in enzyme activity would have to be unusually rapid, since the blocking effect is regularly observed within 1 hour after the onset of acidosis (Table V). There is good evidence that a pH change *per se*, in the range 7.0–7.5, would not greatly alter the activity of carbonic anhydrase (15).

The possibilities of changes in carbonic anhydrase activity in acidosis were explored in heparinized cats under phenobarbital anesthesia. Cats show the same type of renal response to Diamox as dogs, rats, and man; anesthesia does not interfere with the renal response in dogs or cats. The following procedure was used: One kidney was removed and thoroughly perfused with saline to remove blood. The cat was given a 60-minute continuous infusion of HCl totaling 6 meq./kg. Plasma pH dropped to 6.8 and HCO_3^- concentration to 7 meq./l. The second kidney was removed and perfused similarly to the first. Both kidneys were homogenized and analyzed for carbonic anhydrase. The enzyme activity of the two kidneys was the same (360–400 units/ml. in the method of Ref. 14), and was equally inhibited by Diamox.

No evidence has been adduced, therefore, to indicate any change in renal carbonic anhydrase during metabolic acidosis.

2. In metabolic acidosis, the rate of H^+ output is accelerated due either to non-enzymatic hydration of CO_2 or increased H^+ production from other sources. Carbonic anhydrase is thus relegated to a minor role in urinary acidification.

This concept receives support from the fact that the equilibrium under consideration,

$$CO_2 + H_2O \rightleftharpoons H_2CO_3,$$

which is catalyzed by carbonic anhydrase, is also attained fairly rapidly in the *absence* of enzyme. This is evident from the rates obtained for the uncatalyzed forward reaction (14). The forward reaction (either in the presence or absence of enzyme) becomes more rapid as CO_2 is increased, in agreement with the mass action law. Carbonic anhydrase is not necessary for the acidification of urine.[8]

To explain the loss of effect of Diamox in metabolic acidosis, one might suppose that *intracellular* pCO_2 is elevated, and the uncatalyzed reaction speeded. But it should be noted that *plasma* pCO_2 may vary from 25 to 70 mm. Hg, in situations of renal resistance to Diamox (Table VII). Alternatively, it may be postulated that an increase in intracellular H^+ provides an increased gradient for transport of protons from renal tubular cells to the lumen. In both cases (they are the same case if total CO_2 is constant) the role of the enzyme becomes less significant, and the uncatalyzed processes (both the hydration of CO_2 and the H^+ gradient) become more significant. When the enzyme is no longer the rate-controlling factor in urinary acidification the inhibitor will no longer act.

We provisionally conclude that during acidosis, the enzyme becomes relatively unimportant as a rate-controlling factor in urinary acidification. The

TABLE VII

Characteristics of NH_4Cl and Diamox-Induced Acidosis

Plasma	Acute NH_4Cl*	Chronic Diamox†
pH	Low–low normal (7.25)	Low (7.15)
HCO_3^-, meq./l.	Low (12–16)	Low–low normal (12–19)
pCO_2, mm. Hg	Low (25–30)	Low (30), normal or high (70)
Cl^-, meq./ l.	High (115–120)	Low (85), normal or high (120)
Urine		
pH	Low	Normal
Na^+	Normal	Normal
K^+	Moderately high	Normal
Cl^-	Moderately high	Normal
NH_4^+	High normal	Normal
H^+	High normal	Normal

* As exemplified by oral doses of 5 meq./kg., 1 hour before plasma and urine values cited.

† As exemplified by schedules causing loss of approximately 5 meq./kg. of "base" following carbonic anhydrase inhibition [Figs. 1 and 2, and (1)]. Characteristics cited are those after day 1.

uncatalyzed hydration of CO_2, or H^+ (from sources other than CO_2), in the acidotic cell may be considered the chief source(s) of urinary acid.

DISCUSSION

The primary finding is that metabolic acidosis, whether induced by depletion of base or administration of acid as NH_4Cl, reduces the effect of Diamox on renal excretion of HCO_3^-. Such reduction is proportional to the degree of acidosis, as measured by urinary loss of base or the amount of acid given. Secondly, in the setting of metabolic acidosis and renal refractoriness, it was evident that Diamox caused an invariable rise in pCO_2.

A. *Renal*

The questions posed in the introduction may be answered as follows:

1. The components of the acidosis responsible for the renal resistance to Diamox: Since both acute loads of NH_4Cl and repeated doses of Diamox produce this resistance, it is of interest to compare the characteristics of these two types of metabolic acidosis (Table VII). It is evident that these two situations have little in common, except the load of acid given and the amount of base lost, which are chemically equivalent for the host. *The renal resistance appears to be a result of the net change in acid base balance.* The studies cited in Table I, stating that resistance is due to decrease in plasma HCO_3^-, based these conclusions only on the fact that this moiety is decreased following administration of NH_4Cl (6, 7, 8). Two pieces of evidence point away from the role of plasma HCO_3^-: Response to Diamox in respiratory alkalosis with low plasma HCO_3^- of 12 meq./l. (1), and the wide variation in response to Diamox at constant

low plasma HCO_3^- concentrations or filtered loads of HCO_3^-, depending on the amount of base previously lost (Ref. 1: Fig. 11 and present Figs. 1–2).

2. The quantitative conditions to reduce the renal effect: Acid added as NH_4Cl as little as 1 hour before Diamox blocked the renal effect of inhibitor. With maximal inhibition of renal carbonic anhydrase (Diamox at 100 mg./kg.), 2 meq./kg. of prior NH_4Cl did not alter the renal effect, 5 meq./kg. reduced it to about $\frac{2}{3}$, and 12 meq./kg. reduced it to about $\frac{1}{5}$ (Table V). The 2 meq./kg. experiments explain the normal responses to Diamox reported by Berliner *et al.* (3, 4) in "acidotic dogs" (see Table I). They also indicate that NH_4Cl and Diamox, in certain dose combinations, can produce a greater acidosis than the given dose of either agent alone. Since 2 meq./kg. of NH_4Cl did not affect the renal response to Diamox at 100 mg./kg., the ensuing acidosis must inevitably be greater than that due to Diamox alone, by just the dose of acid added. This principle was used by Rubin *et al.* (16) to produce a clinical acidosis in refractory cardiac failure prior to administration of a mercurial diuretic.

NH_4Cl added after the onset of Diamox diuresis can completely abolish that effect within 2 hours. The renal resistance produced by Diamox itself takes much longer to develop, at least 12–24 hours. But it seems reasonable to believe that this is merely due to the fact that even under conditions of maximum inhibition, it takes that long for the dog to lose about 5 meq./kg. of base (Figs. 1–2). Whether secondary effects in response to the loss of base, possibly mediated by the adrenal cortex, come into play at this time remains to be determined. On the premise that net body metabolic acidosis produced by Diamox and NH_4Cl are the same[9]: *the blocking of maximal doses of Diamox appears independent of the length of acidosis, but requires 5–12 meq./kg. prior loss of base or gain of acid.*

3. In the setting of near maximal carbonic anhydrase inhibition, the changes in HCO_3^- excretion in the normal and in the acidotic dog: In the normal dog, maximal inhibition of carbonic anhydrase diverts approximately 20 per cent of filtered HCO_3^- into the urine.[3] In severe metabolic acidosis, inhibition of enzyme diverts but 3–6 per cent (and occasionally none) of filtered HCO_3^- into the urine. In the latter situation, therefore, *the role of the enzyme is reduced to about $\frac{1}{5}$ of what it is normally.* Calculation of the effect of Diamox in terms of net base excretion lost ($NH_4^+ + H^+ - HCO_3^+$) indicates a similar reduction (to $\frac{1}{4}$) in the latter situation in metabolic acidosis. Effects on Na^+ are similar to those of HCO_3^-. With partial blocking of the Diamox effect, there is a tendency toward greater excretion of K^+ than of Na^+.

4. Explanation for renal resistance to Diamox in acidosis: Neither the concentration of renal carbonic anhydrase, nor its chemical capacity to respond to

[9] Further support that this is the case comes from the finding (Maren, T. H., Fed. Proc., 1955, **14:** 366) that acidosis due to Diamox potentiates mercurial diuretics, in similar fashion to acidosis following NH_4Cl or NH_4NO_3.

Diamox, is altered in severe metabolic acidosis. The pharmacology of Diamox is but slightly changed in acidosis. *The data suggest that in acidosis the participation of carbonic anhydrase in renal acidification is diminished.* Unlike most biological systems under enzymatic control, the uncatalyzed hydration of CO_2 has a very appreciable rate *in vitro* (15); increase in CO_2 pressure increases rate (14). As noted above (Section C) either increase in CO_2 or in another metabolic source of H^+ would increase urinary acidity without carbonic anhydrase.

Insofar as the data permit comparison, the present studies in the dog agree well with those done in man, both on effects of Diamox alone (8) and those following NH_4Cl acidosis (9).

Two final points may be emphasized. First, the role of carbonic anhydrase is quantitatively small (20 per cent) in normal renal bicarbonate reabsorption. The decrease of this role in acidosis (to about 4 per cent) does not require that any new process arise; the carbonic anhydrase independent system(s) need only increase participation in HCO_3^- reabsorption from 80 to 96 per cent to account for the observed results. Secondly, even massive acidosis does not completely abolish the effect of intravenous or very high oral doses of Diamox. The relationships are quantitative. The effects of acidosis on the response to Diamox may best be gauged by comparison, preferably in the same animal, to responses obtained under conditions of normal acid-base balance.

B. *Respiratory*

It is evident that Diamox tends to produce an elevation in plasma pCO_2 but the effect may be masked or reversed by a decrease in blood HCO_3^- following renal excretion of HCO_3^-. However, elevation of pCO_2 is uniformly observed when the renal effect of Diamox is blocked. There were no striking changes in breathing during Diamox treatment of an acidotic (due to NH_4Cl) dog.[10] Simultaneous measurements of respiration, renal function and acid-base balance are required to document the sequence of events. Some studies of this type have been done following Diamox alone; data are not entirely consistent. In acute studies, E. L. Becker *et al.* (17) could not demonstrate a rise in pCO_2 following large intravenous doses of Diamox to patients without pulmonary disease. Tomashefski *et al.* (18), Bell *et al.* (19) and Shepard *et al.* (20) showed

[10] It is well established for both unanesthetized dogs and man that slight increments in arterial blood pCO_2 are associated with large increases in minute ventilation. In previous studies (1) we have ruled out unusual differences between arterial and venous pCO_2 following Diamox.

It is therefore of interest that despite increase in blood pCO_2 of as great as 20 mm. Hg, changes in ventilation were clinically undetectable. We are indebted to Dr. Alfred P. Fishman for pointing out this anomaly.

small rises of arterial blood pCO_2 following oral Diamox in man; in the last-named study the effect could only be elicited by exercise. Cranston *et al.* (21) found an initial rise in arterial but not alveolar pCO_2 following oral Diamox in man, with a later fall in alveolar pCO_2 when diuresis had been established. In anesthetized dogs (18) there was also a rise in arterial blood but not alveolar pCO_2 after Diamox. The gradient between arterial and alveolar pCO_2 following Diamox has not been satisfactorily explained (18–21). In chronic studies, on schedules of Diamox that produce metabolic acidosis, Nadell (22) and Galdston (23) both found lowering of pCO_2 in man; this is also seen in certain regimens in the dog (1). It would be of interest to study respiratory effects of Diamox in man, after blocking of the renal action.

The present studies suggest that following Diamox administration alone, the metabolic acidosis due to loss of base tends to compensate (in terms of pCO_2) for the respiratory acidosis, which may be due to inhibition of blood carbonic anhydrase (2). As a result, pCO_2 is usually close to normal following Diamox. When either of the types of acidosis is eliminated, the effect of the other is unmasked, leading to predictable changes in pCO_2.

CONCLUSION

Metabolic acidosis in dogs was induced by administration of Diamox (base loss) or by oral NH_4Cl (acid gain). Either base loss or acid gain in the range 5–12 meq./kg. diminished or abolished the typical renal effects of Diamox on HCO_3^- excretion.

Experiments with large doses of Diamox, in order to approach 100 per cent inhibition of renal carbonic anhydrase, indicated that this enzyme is the rate-controlling factor in about 20 per cent of HCO_3^- reabsorption in the normal dog. In the acidotic dog, this is reduced to about 4 per cent. It appears that the role of renal carbonic anhydrase is diminished in acidosis. We suggest that in acidosis the uncatalyzed hydration of CO_2, or H^+ from another source, can furnish urinary acid at a rate which makes the enzymatic process appear negligible. Renal carbonic anhydrase, and its *in vitro* response to Diamox, is unaltered in metabolic acidosis due to HCl.

When the renal effect of Diamox is reduced by induction of metabolic acidosis, the effect of the inhibitor in elevating blood pCO_2 is uncovered

ACKNOWLEDGMENT

We wish to thank Miss Clara Pellicci and Miss Lena Bioletti of these Laboratories for carrying out many of these experiments. Drs. Robert W. Berliner, Jackson P. English, John T. Litchfield, Jr. and George M. Sisson made valuable suggestions during the preparation of the manuscript.

REFERENCES

1. Maren, T. H., Wadsworth, B. C., Yale, E. K. and Alonso, L. G.: Carbonic anhydrase inhibition. III. Effects of Diamox® on electrolyte metabolism. Bull. Johns Hopkins Hosp., 1954, **95:** 277.
2. Maren, T. H., Mayer, E. and Wadsworth, B. C.: Carbonic anhydrase inhibition. I. The pharmacology of Diamox® 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide. Bull. Johns Hopkins Hosp., 1954, **95:** 199.
3. Berliner, R. W., Kennedy, T. J. and Orloff, J.: Relationship between acidification of the urine and potassium metabolism. Am. J. Med., 1951, **11:** 274.
4. Berliner, R. W.: Renal secretion of potassium and hydrogen ions. Fed. Proc., 1952, **11:** 695.
5. Schwartz, W. B., Danzig, L. E. and Relman, A. S.: Role of carbonic anhydrase in renal tubular reabsorption of bicarbonate. Am. J. Med., 1953, **14:** 526.
6. Leaf, A., Schwartz, W. B. and Relman, A. S.: Oral administration of a potent carbonic anhydrase inhibitor ("Diamox"). I. Changes in electrolyte and acid-base balance. New England J. Med., 1954, **250:** 759.
7. Madsen, P. O.: The effect of the carbonic anhydrase inhibitor, Diamox, on urine electrolytes in normal and adrenalectomized dogs, and its possible relation to serum bicarbonate. Acta physiol. Scandinav., 1954, **31:** 28.
8. Counihan, T. B., Evans, B. M. and Milne, M. D.: Observations on the pharmacology of the carbonic anhydrase inhibitor "Diamox". Clin. Sc., 1954, **13:** 583.
9. Kaye, M.: The effect of a single oral dose of the carbonic anhydrase inhibitor, acetazolamide, in renal disease. J. Clin. Invest., 1955, **34:** 277.
10. Jenson, R. L., Tobias, G. J., Greaney, J. F., Relman, A. S. and Schwartz, W. B.: Renal tubular function during severe metabolic acidosis. Am. J. Physiol., 1954, **179:** 188.
11. Schwartz, W. B. and Relman, A. S.: The dependence of renal sodium reabsorption on hydrogen exchange. J. Clin. Invest., 1954, **33:** 965.
12. Giotti, A. and Maynert, E. W.: The renal clearance of barbital and the mechanism of its reabsorption. J. Pharmacol. & Exper. Therap., 1951, **101:** 296–309.
13. Hodler, J. E., Heinemann, H. O., Fishman, A. P. and Smith, H. W.: Urine pH and carbonic anhydrase activity in the marine dogfish. Am. J. Physiol., 1955, **183:** 155.
14. Maren, T. H., Ash, V. I. and Bailey, E. M., Jr.: Carbonic anhydrase inhibition. II. A method for determination of carbonic anhydrase inhibitors, particularly of Diamox®. Bull. Johns Hopkins Hosp., 1954, **95:** 244.
15. Roughton, F. J. W.: Some recent work on the chemistry of carbon dioxide transport by the blood. Harvey Lectures 1943–44, p. 118.
16. Rubin, A., Thompson, H. G., Braveman, W. and Luckey, E. H.: The management of refractory edema in heart failure. Ann. Int. Med., 1955, **42:** 358.
17. Becker, E. L., Hodler, J. E. and Fishman, A. P.: Effect of carbonic anhydrase inhibitor (6063) on arterial-alveolar CO_2 gradient in man. Proc. Soc. Exper. Biol. & Med., 1953, **84:** 193.
18. Tomashefski, J. F., Chinn, H. I. and Clark, R. T., Jr.: Effect of carbonic anhydrase inhibition on respiration. Am. J. Physiol., 1954, **177:** 451.
19. Bell, A. L. L., Smith, C. N. and Andreae, E.: Effects of the carbonic anhydrase inhibitor "6063" (Diamox) on respiration and electrolyte metabolism of patients with respiratory acidosis. Am. J. Med., 1955, **18:** 536.

20. SHEPARD, R. H., DONOSO, H., KILLICH, E. M., CHERNIACK, R. M., JOHNS, C. J. AND RILEY, R. L.: Interference with release of CO_2 from pulmonary capillary blood after inhibition of carbonic anhydrase. Fed. Proc., 1954, **13:** 135.
21. CRANSTON, W. I., SANDERSON, P. H. AND STAPELTON, T.: The effects of acetazoleamide on CO_2 carriage in man. J. Physiol., 1955, **129:** 71P.
22. NADELL, J.: The effects of the carbonic anhydrase inhibitor "6063" on electrolytes and acid base balance in two normal subjects and two patients with respiratory acidosis. J. Clin. Invest., 1953, **32:** 622.
23. GALDSTON, M.: Respiratory and renal effects of a carbonic anhydrase inhibitor (Diamox) on acid-base balance in normal man and in patients with respiratory acidosis. Am. J. Med., 1955, **19:** 516.
24. MAREN, T. H.: Carbonic anhydrase inhibition. V. The metabolic conversion of N^5-substituted 2-acetylamino-1,3,4-thiadiazole-5-sulfonamides. In preparation.

HEREDITARY ELLIPTOCYTOSIS AND HEMOGLOBIN C TRAIT

A Report of Two Cases

MARY ELLEN AVERY

Department of Pediatrics, The Johns Hopkins University School of Medicine and the Harriet Lane Home of the Johns Hopkins Hospital

Received for publication October 10, 1955

Chance will favor the combination of elliptocytosis and hemoglobin C trait only rarely in a general population. Hereditary elliptocytosis is an anomaly of red cell shape which is usually considered a benign disorder. It is known, however, to be associated with a hemolytic anemia in some cases. Hemoglobin C is one of the abnormal hemoglobins which is detected by its rate of migration in an electric field. Individuals who are heterozygous with respect to this hemoglobin are asymptomatic, and usually discovered on routine screenings.

These two hereditary abnormalities, one of red cell shape, the other of hemoglobin, have been found in combination in two siblings. Neither of these patients would have been studied for this combination of disorders were it not that a third sibling with elliptocytosis was found to have mild hemolytic anemia. This latter patient had a normal hemoglobin electrophoretic pattern and has been described elsewhere (1). In the investigation of other members of this family, hemoglobin C trait was found in the mother, and hemoglobin C trait and elliptocytosis together were found in the two children who are the subject of this report.

One might anticipate that an abnormal hemoglobin which in the homozygous state may cause a mild hemolytic anemia, in association with a trait giving an abnormal shape to the red cell and in itself capable of producing increased hemolysis, would in combination produce a hemolytic disease. Such does not appear to be the case.

METHODS

The hemograms, blood groups, and the electrophoretic patterns of the hemoglobin were determined on the blood of all available members of three generations of a Negro family in order to demonstrate the genetic pattern of the elliptocytosis and hemoglobin C trait in this family.

The two patients reported in detail were followed by serial hemograms. Reticulocyte counts were done by the wet brilliant cresyl blue technique. Platelets were counted by the phase microscopy technique (2). Hemoglobin electrophoretic mobility was determined by filter paper electrophoresis (3). Red cell survival was studied using radioactive chromium 51 (4). Osmotic and mechanical fragility of the red cells, with and without prior incubation, were measured (5). Serum iron values were also determined (6).*

* Drs. Ernest Smith, William Zinkham, and Charles Sinn of the Department of Medicine studied the hemoglobin electrophoresis, osmotic and mechanical fragilities, and red cell

TABLE I

Summary of Hematologic Data

Patient	Ch. A. (father)	E.A. (mother)	Ce.A.	C.A. (Case II)	O.A. (Case I)	F.A.
Age	30 yrs.	29 yrs.	10 yrs.	7 yrs.	2 yrs.	15 mos.
Blood type	(A_1M Rh_0 positive)	(OMN Rh^1 hr^1 positive)	(OM Rh_0)	(OM Rh_0)	(OM Rh_0hr^1 positive)	(A_1M Rh_0)
RBC million per c.mm.	4.87	4.44	4.72	4.75	5.82	5.67
Hgb. gms. per 100 cc.	13.6	11.1	12.3	11.1	9.8	9.1
Hematocrit	43.8	33.0	40.0	36.8	33	32.1
Reticulocyte %	1.7	2.0	0.8	1.7	2.8	2.4
MCV in C.$\mu\mu$	89	74	85	77	57	58
MCH in $\mu\mu$g.	28	25	30	23	17	16
MCHC %	31	34	31	30	30	28
Sickle prep. (2% sodium metabisulfite)	—	Neg.	Neg.	Neg.	Neg.	Neg.
WBC per c. mm.	3,650	6,700	5,300	8,600	13,650	10,650
Platelet count	185,000	350,000	345,000	463,000	424,000	508,000
Hgb. electrophoresis	Normal	C trait	Normal	C trait	C trait	Normal
Elliptocytes	Present	Absent	Absent	Present	Present	Present
Mechanical fragility %						
Non-incubated	1.4	1.2		3.1	2.0	6.0
Incubated	3.3	7.6		2.5	9.7	9.6

CASE REPORTS

Case I. O. A. is a Negro female who was born November 15, 1952 with a birth weight of 3400 grams following an uneventful, term pregnancy. She had no neonatal jaundice. At age two weeks she was first seen in the Harriet Lane Home because of a mild upper respiratory infection, and at that time appeared to be a well developed and active infant. She was seen frequently in subsequent months because of diarrhea and minor infections, and it was noted that her developmental pattern was normal. At age eighteen months she weighed 11 kilograms. At this time her mother said she had been eating plaster. A blood lead level was found to be 0.047 mg. per cent (within the range of normal); however, a month later it was 0.086 mg. per cent, and increased urinary coproporphyrins were found. Hematologic studies were first performed in July 1954 when she was twenty months old, and revealed a hemoglobin of 6.6 grams (Table II). A few elliptocytes and target cells were noted on the smear (see Fig. 1). She had a poor appetite and had refused to eat solids. Treatment was started

life span respectively. Dr. Hugh Josephs of the Department of Pediatrics determined the serum iron levels.

TABLE II

Case I—Summary of Data

Date	Age	Wt.	RBC	Hgb.	Hct.	MCV	MCH	MCHC	Serum Iron	Reticulocyte percentage	Lead Level	Comment
	mos.	*kg.*							*micrograms %*		*mg. %*	
7-23-54	20	12	4.55	6.6	27	59	14	22			.086	Given iron
9-22-54	22		6.04	9.6	34	56	16	28	78	1.8	.080	
10- 7-54			4.84	9.3	30	62	19	31	80	.97		
10-22-54	23		5.34	9.9	33	62	18	30	79	.8	.059	
12-17-54		13.7	6.37	12.5	38.5	60	20	33	141	.2		Iron discontinued
1-17-55			5.30	11.4	37	70				1		
2-11-55	27		—	10.2	32	—			63	.3		
3-3-55			5.82	9.8	33	57	17	30		2.8		

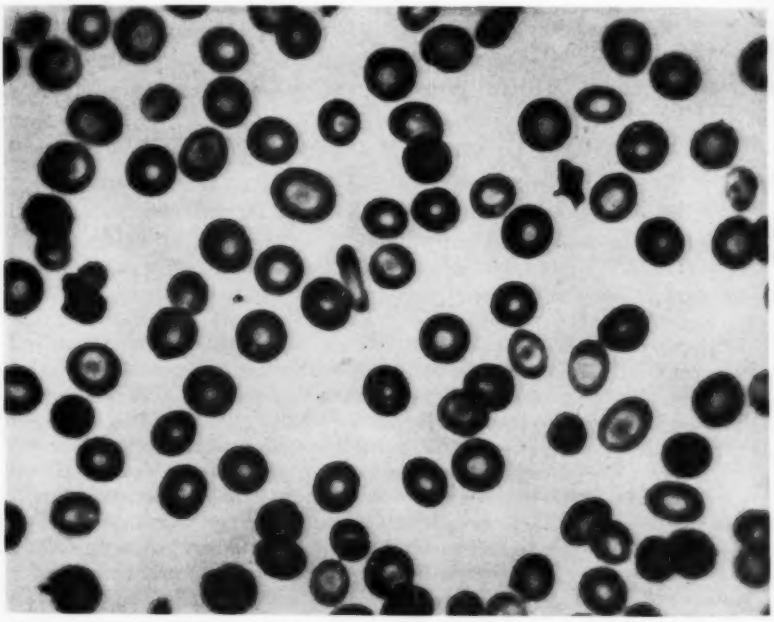


FIG. 1. Case I. O. A. Hemaglobin C and elliptocytosis. This smear was made after iron therapy and cessation of plaster ingestion.

with ten per cent ferric ammonium citrate, given orally, one teaspoon three times a day. Two months later, her hemoglobin was 9.6 grams. The blood lead level was still elevated to 0.080 mg. per cent, but one month later it had fallen to 0.059 mg. per cent and her peripheral blood picture was unchanged. Iron therapy was discontinued after five months, and she has maintained her hemoglobin at about 10 grams. Her smear has consistently shown some elliptocytes and many target cells.

TABLE III

Case II—Summary of Data

Date	Age	Wt.	RBC	Hbg.	Hct.	MCV	MCH	MCHC	Reticulocyte Percentage	Comment
		kg.								
6-28-50	3 yrs., 2 mos.	16		7.0						Started iron
8-18-50				10.8						Iron discontinued
9-22-54			4.43	11.4	37	84	25	30	0.1	
2-24-55	7 yrs.	27	4.45	12.1	36	82	27	33	0.7	
3-3-55			4.75	11.1	36.8	77	23	30	1.7	

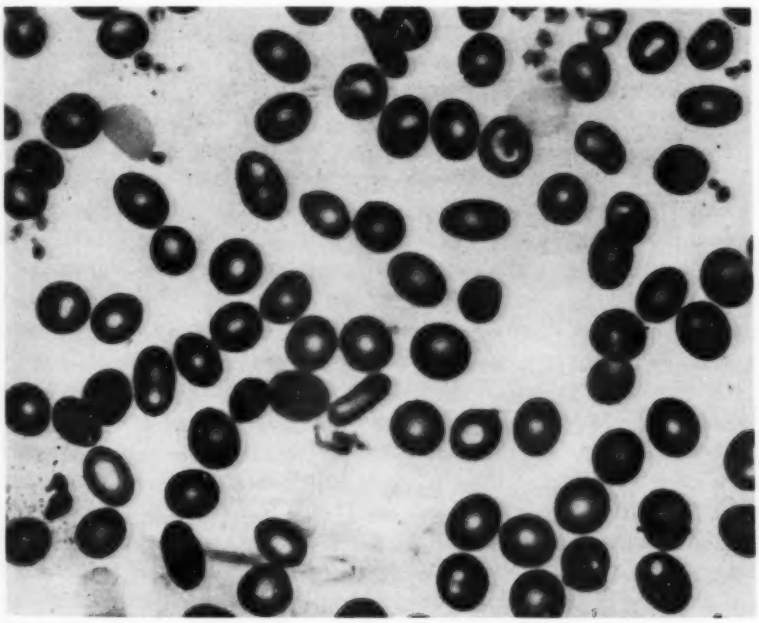


FIG. 2. Case II. C. A. Hemoglobin C trait and elliptocytosis. Note the elliptical target cells upper left part of field.

At age two years she was 91 centimeters tall and weighed 13.7 kilograms. There was no scleral icterus. Examination of her ears, nose, and throat revealed no focus of infection. Her heart was normal size and no murmurs were heard. The liver and spleen were not palpable. Her extremities were of average proportions.

Case II. C. A. is a Negro male who was born April 17, 1947, with a birth weight of 4100 grams. He had no neonatal jaundice or bleeding. He was breast fed, and developed normally. In February 1950 he was first seen in the Harriet Lane Home because of a respiratory infection, and weighed 15 kilograms. At age three years and two months, in June 1950, he was noted to be pale, but not icteric. A sickle cell preparation was negative. Because of a hemoglobin of 7.0 grams he was given oral ferric ammonium citrate, one teaspoon three times a day, and two months later his hemoglobin was 10.8 grams (Table III). He was next seen in September 1954 at age seven years and five months, when hematologic studies of his family were undertaken. His hemoglobin was 11.4 grams, and his smear showed some elliptical cells (Fig. 2). He had in addition the electrophoretic pattern of hemoglobin C trait. He was a well developed boy who weighed 27 kilograms and was 130 centimeters tall. Physical examination was within normal limits. He had no hepatosplenomegaly, icterus, or chronic infection.

DISCUSSION

I. Elliptocytosis

Elliptical red cells have been described as a harmless morphologic anomaly (7–11) as well as in association with mild compensated hemolysis or with a hemolytic anemia (12, 13). Heilmeyer's classification seems useful (18). He suggests three categories: 1) Hereditary elliptocytosis with no signs of hemolysis; 2) hereditary elliptocytosis with hemolysis but no anemia; 3) hereditary elliptocytosis with hemolytic disease and anemia.

The evidence for considering the anomaly of the cell the responsible factor in hemolysis is abundant. Huck and Bigelow demonstrated in 1923 that the washed elliptic cells were not altered after three weeks in normal serum, nor did normal red cells become elliptic after an equal time in the serum of an elliptocytic patient (15). The work of Berlin and Hedenstedt in 1952 using two methods to determine cell survival time, suggested that the elliptocytes reverted to normal shape in a recipient's circulation (17). Using the differential agglutination method they determined a half life of forty-seven days, whereas with serial microphotographs following the percentages of transfused elliptocytes after a given time interval, the half life was fifteen days. The work of Motulsky *et al.* (13) did not confirm these discrepancies. Using both the Ashby and the visual counting technique, they found good agreement in studying the red blood cell life span of three patients with elliptocytosis. In two of these patients, the life span was normal. A third had a red cell life span of forty-five days, and this patient also had evidence of increased hemolysis in the absence of anemia.

There are a number of reports of elliptocytosis in association with evidence of hemolysis (1, 12–14). Lipton has recently reported a case of an infant both

of whose parents have elliptocytes (19). The infant was found to have a marked hemolytic anemia at one month of age, and a decreased red cell life span. He was much improved by splenectomy at age seven months. Wyandt, Bancroft, and Winship (7) reported the first such infant, thought to be homozygous with respect to the elliptocytic red cell trait. This child also had a hemolytic anemia, with splenomegaly.

It has been amply demonstrated that siblings may be affected in varying degrees in the heterozygous state with respect to elliptocytosis (1). In the homozygous state, apparently there is a much more severe condition (19). Motulsky *et al.* have compared this to the situation which exists in thalassemia minima and thalassemia minor, and the homozygous thalassemia major (13). They suggest an alternate hypothesis, namely some genetic defect not now detectable may be acting in association with the gene for elliptocytosis to alter its effect.

II. Hemoglobin C

The finding of an abnormal hemoglobin associated with sickle cell trait and disease by Pauling *et al.* in 1949 (20) heralded a host of investigations into hemoglobin types. Itano and Neel (21) reported in 1950 another hemoglobin type, subsequently called hemoglobin C. This has been studied in association with the hemoglobin of sickle cell disease (22), giving SC disease, and also in the homozygous or CC combination (22–24).

Hemoglobin C trait has been found exclusively in Negroes, and is less common than hemoglobin S trait. Smith and Conley have found the incidence of hemoglobin C trait in Negroes in Baltimore to be about two per cent (3). It appears to be transmitted as a Mendelian dominant (3). Individuals with the combination of normal (A) and hemoglobin C, (AC), do not have an associated anemia. The diagnosis may be suspected on the basis of an increased number of target cells in the peripheral blood, and confirmed only by determining the electrophoretic pattern of the hemoglobin. Patients with homozygous hemoglobin C disease may have a mild anemia, and on further study may demonstrate a compensated hemolytic process. The case studied by Spaet *et al.* had a hematocrit value which varied between 30–39 per cent, and a minimal reticulocytosis (23). The red cell life span in this case was 55 days using the Ashby technic. Some patients with homozygous C disease have been reported as asymptomatic, others apparently have some arthralgia or abdominal cramps (24). Splenomegaly may or may not be present.

COMMENT ON CASES

The two children presented as having hereditary elliptocytosis and hemoglobin C trait were discovered during a family study of a patient with marked elliptocytosis and increased hemolysis. Case I (O. A.) was anemic, though her

anemia could have been secondary to plumbism and iron deficiency. The rise in her hemoglobin from 6.6 grams to 9.6 grams after two months of iron therapy suggests that iron deficiency was an important factor. There has not been a persistent reticulocytosis, and the red cell survival using radioactive Cr 51 was within the range of normal.

Case II (C. A.) has a hemogram which is normal for a seven year old Negro male. At age 3 years he had a low hemoglobin, but after two months of iron therapy, his hemoglobin was 10.8 grams. There were no additional studies done at that time. However, from the history there is no reason to believe he had any hemolytic episodes. These children appear to have no abnormalities which can be attributed to their hematologic anomalies.

The father has about 15 per cent elliptical cells, and is not anemic (Table I). The mother has no elliptocytes, but carries hemoglobin C trait. She was pregnant at the time she was studied, and it is suggested this may account for her slight anemia. The oldest daughter has neither elliptocytosis nor any abnormality of her hemoglobin on electrophoresis. The youngest child (F. A.)

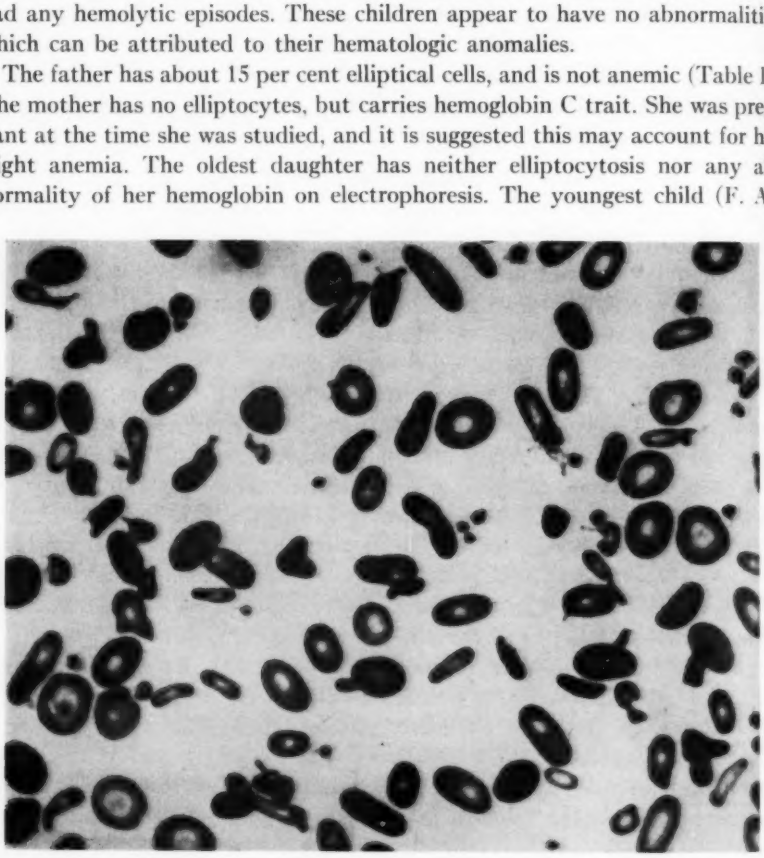


FIG. 3. F. A. Note the bizarre forms, and characteristic concentration of hemoglobin at either pole of the elliptocyte. This patient has normal hemoglobin by paper electrophoresis. Josephs, H. W. and Avery, M. E.: Hereditary elliptocytosis associated with increased hemolysis. Pediatrics, 1955, **16**: 741. Reproduced, courtesy of Charles C. Thomas, Publisher, Springfield, Illinois.

has a marked elliptocytosis and a normal electrophoretic hemoglobin pattern (Fig. 3). This child has a red cell count of about five million, and a hematocrit of 30 to 35. He also has a consistently elevated reticulocyte count, about 2–6 per cent. His red cell life span using radioactive chromium 51 is decreased (see chart), indicative of a mild and well-compensated hemolytic tendency (1).

Diagnosis

Florman and Wintrobe have set up numerical standards for the diagnosis of hereditary elliptocytosis (11). The father of this family would not meet their criteria for the diagnosis since he has only fifteen per cent elliptical cells. However, three out of four of his children studied have elliptocytes, and one (F. A.) has a mild hemolytic anemia attributed to his elliptocytosis.

This family demonstrates the fallacy of trying to assign quantitative values to the cells necessary to make the diagnosis of hereditary elliptocytosis. The criteria for diagnosis on the basis of careful measurements of cell diameters are of much less value than the study of members of the family.

Motulsky *et al.* (13) have suggested a useful descriptive classification based on the numbers of round, oval, elliptical, and rod forms (Fig. 5). This is helpful

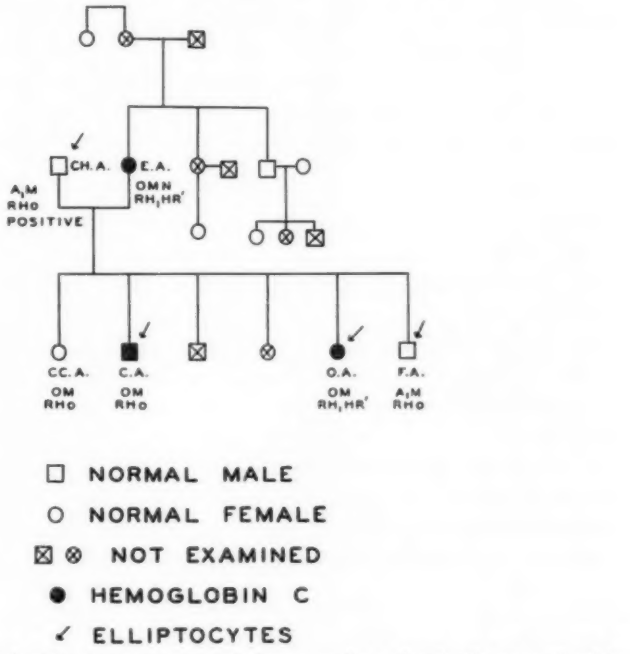


FIG. 4. Family study. The two cases presented are members of a family as outlined in the family tree.

CASE 1 O.A.	82%	12%	4%	2%	21%
CASE 2 C.A.	50%	24%	20%	6%	14%

FIG. 5. Distribution of the degree of elliptocytosis (at least 400 cells counted, using dried smears of capillary blood). This is an arbitrary division, as used by Motulsky, Singer, Crosby and Smith (13).

in estimating the severity of the condition, but has no place in establishing the diagnosis. In these cases, the per cent of the total number of cells which are target cells is also tabulated. Many of the oval and elliptical cells are also targeted.

Elliptical target cells

Hereditary elliptocytosis has been described in association with sickle cell trait by Fadem in a 25 year old Negro male (25). It is of interest that in this case the elliptocytes were not observed to sickle. In the cases presented here, some of the target cells, associated with hemoglobin C trait, were elliptical (Fig. 2). This is evidence that some of the elliptical cells contain hemoglobin C, and that the two genetic effects are thus manifest in the same cell.

Genetic aspects

Goodall *et al.* have reported a family with elliptocytosis in six members, and absent in twelve members (26). All the affected individuals were type cDE (Rh_2) and none of the unaffected members possessed this gene complex. They concluded that in this family there was no doubt that the gene determining the oval cell shape was on the same chromosome as the gene controlling the Rh blood group. Chalmers and Lawler studied two other families, and found no close linkage of the genes for elliptocytosis and any of the blood group systems (27).

In the family presented above, there is no apparent linkage of elliptocytosis and blood groups. The father (Ch. A.) has elliptocytosis and is group A_1MRh_0. One child with elliptocytosis has the identical blood group; however, another child with elliptocytosis has blood group $OMRh_1HR^1$.

Fragility studies

The red cell fragility tests in these cases are of interest especially because of the abnormality accentuated by incubation for twenty-four hours at 37° centi-

grade (Fig. 6). In cases of congenital spherocytosis the shift in the direction of increased fragility is enhanced by incubation, and in some cases, may not be apparent at all in the non-incubated blood (28).

In the cases presented here, the osmotic fragility curves using fresh defibrinated blood are in or near the normal range; however, after incubation for twenty-four hours at 37° centigrade, the curves are shifted in the direction of increased resistance. The patient with extreme elliptocytosis (F.A.) has a markedly abnormal curve, while the patient with mild elliptocytosis (Ch.A., father) has a normal fragility pattern. The patient with C trait alone (E.A., mother) has somewhat increased resistance in the incubated blood.

The exact mechanism and significance of this effect of incubation is not clear, but it would appear to be a more sensitive method of demonstrating *in vitro* fragility of the red cells than the older technique of using fresh blood. The mechanical fragility of the red cells is increased after incubation, markedly so in Case I and less so in Case II. There is no obvious explanation for this.

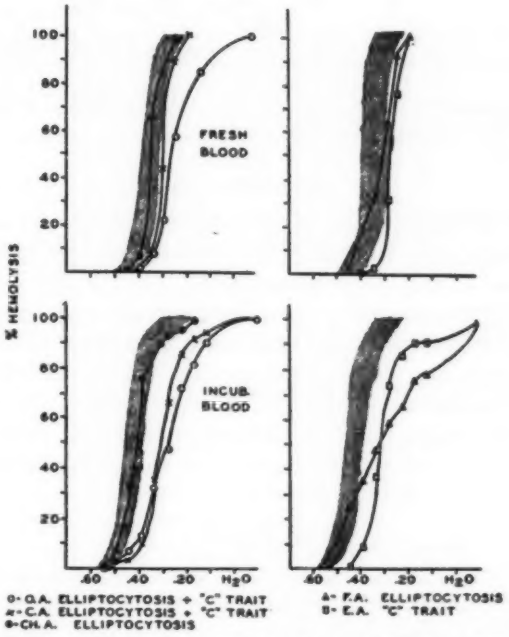


FIG. 6. The osmotic fragility curves of freshly obtained defibrinated blood and incubated defibrinated blood are shown above. The curves obtained with fresh blood are in or near the normal range. The curves of incubated blood of the C trait and elliptocytosis patients are displaced in the direction of increased resistance.

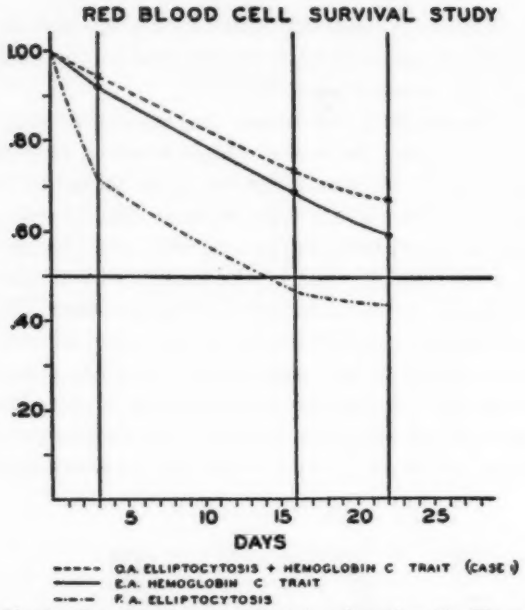


FIG. 7. Red blood cell survival using radioactive chromium 51. The normal half life of the red cells by this technique is twenty-eight days.

Red cell survival

The red cell life spans of one of the patients presented (Case I), a sibling with elliptocytosis and a well-compensated hemolytic anemia, and the parent with hemoglobin C trait alone were studied (Fig. 7). The patient with the marked elliptocytosis showed a diminished red cell survival, with approximately one-half his cells surviving at fourteen days. With this method, a half life of twenty-eight days is considered normal (4). The patient with mild elliptocytosis plus hemoglobin C trait, on the other hand had a normal red cell survival time, as did the parent with hemoglobin C trait alone. This is additional evidence that the combined anomalies are not necessarily additive in effect.

SUMMARY

1. The clinical and hematologic findings of two patients who manifest both hereditary elliptocytosis and hemoglobin C trait are presented.

2. The findings in other members of the family as they pertain to these two abnormalities are outlined.

3. Elliptocytosis and hemoglobin C trait in association do not produce a summation of effect. These patients show no clinical stigmata which can be related to their hematological abnormalities.

4. A correlation is suggested between the severity of elliptocytosis as seen on the blood smear and the hematologic effect in the patient.

5. The red cell life span as determined by radioactive chromium 51 was normal in the patient with elliptocytosis and hemoglobin C trait, normal in the mother with hemoglobin C trait alone, and shortened in a sibling with severe elliptocytosis alone.

6. Osmotic fragility studies on fresh and incubated blood showed increased resistance of the incubated specimens in the patients with elliptocytosis and hemoglobin C trait. Both anomalies alone also gave some increased resistance in the incubated specimens.

7. There was no evidence of linkage of the gene for elliptocytosis with the blood group systems in this family.

BIBLIOGRAPHY

1. Josephs, H. W. and Avery, M. E.: Hereditary elliptocytosis associated with increased hemolysis. Pediatrics, 1955, **16:** 741.
2. Brecher, G. and Cronkite, E. P.: Morphology and enumeration of human blood platelets. J. Appl. Physiol. 1950, **3:** 365.
3. Smith, E. W. and Conley, C. L.: Filter paper electrophoresis of human hemoglobins with special reference to the incidence and clinical significance of hemoglobin C. Bull. Johns Hopkins Hosp. 1953, **93:** 94.
4. Necheles, T. F., Weinstein, I. M. and LeRoy, G. V.: Radioactive sodium chromate for the study of survival of red blood cells. J. Lab. and Clin. Med. 1953, **42:** 358.
5. Ham, T. H.: *A Syllabus of Laboratory Examinations in Clinical Diagnosis*. Harvard University Press, Cambridge, Mass. 1950.
6. Josephs, H. W.: Determination of iron in small amounts of serum and whole blood with the use of thiocyanate. J. Lab. and Clin. Med. 1954, **44:** 63.
7. Wyandt, H., Bancroft, P. M. and Winship, T. O.: Elliptic erythrocytes in man. Arch. Int. Med. 1941, **68:** 1043. (Review)
8. Cheney, G.: Elliptic human erythrocytes. J. Am. Med. Assn. 1932, **98:** 879.
9. Stephens, D. J. and Tatelbaum, A. J.: Elliptical human erythrocytes. J. Lab. and Clin. Med. 1935, **20:** 375.
10. McCarty, S. H.: Elliptical red blood cells in man—a report of eleven cases. J. Lab. and Clin. Med. 1934, **19:** 612.
11. Florman, A. L. and Wintrobe, M. W.: Human elliptical red corpuscles. Bull. Johns Hopkins Hosp. 1938, **63:** 209.
12. Giffin, H. Z. and Watkins, C. A.: Ovalocytosis with features of hemolytic icterus. Tr. Assn. Am. Physn. 1939, **54:** 355.
13. Motulsky, A. G., Singer, K., Crosby, W. H. and Smith, V.: The life span of the elliptocyte. Blood 1954, **9:** 57.
14. Trinick, R. H.: Elliptocytosis. Lancet 1948, **1:** 963.
15. Huck, J. G. and Bigelow, R. M.: Poikilocytes in otherwise normal blood. (elliptical human erythrocytes) Bull. Johns Hopkins Hosp. 1923, **34:** 390.
16. Mason, V. R.: Ovalocytosis (elliptical human erythrocytes). *Handbook of Hematology*, Vol. 3 edited by H. Downey, Paul Hoeber, New York, N. Y., pp. 2351–2360, 1938.
17. Berlin, R. and Hedenstedt, S.: The life span of elliptocytes. Acta med. Scand. 1952, **143:** 273.

18. Heilmeyer, L. and Begemann, H.: Blut und Blutkrankheiten (ed. 4 of *Handbuch der inneren Medizin*). Springer Verlag, Berlin, Germany 1951, vol. 2 p. 334, (cited by Motulsky et al. Blood 1954, **9:** 57.
19. Lipton, E. L.: Elliptocytosis with hemolytic anemia: The effects of splenectomy. Pediatrics 1955, **15:** 67.
20. Pauling, L., Itano, H. A., Singer, S. J. and Wells, I. C.: Sickle cell anemia, a molecular disease. Science 1949, **110:** 543.
21. Itano, H. A. and Neel, J. V.: A new inherited abnormality of human hemoglobin. Proc. Nat. Acad. Sc. 1950, **36:** 613.
22. Kaplan, E., Zuelzer, W. W. and Neel, J. V.: Further studies on hemoglobin C. II. The hematologic effects of hemoglobin C alone and in combination with sickle cell anemia. Blood 1953, **8:** 735.
23. Spaet, T. H., Alway, R. H. and Ward, G.: Homozygous type "C" hemoglobin. Pediatrics 1953, **12:** 483.
24. Morgan, J. L., Bowles, R. M. and Harris, J. S.: Hemoglobin C. Report of the homozygous condition and of combination with normal and sickle cell hemoglobin. Pediatrics 1955, **15:** 185.
25. Fadem, R. S.: Ovalocytosis associated with the sickle cell trait. Blood 1949, **4:** 505.
26. Goodall, H. B., Hendry, D. W. W., Lawler, S. D. and Stephens, S. A.: Data on linkage in man: Elliptocytosis and blood groups II, family 3. Ann. Eugen. 1953, **17:** 272.
27. Chalmers, J. N. M. and Lawler, S. D.: Data on linkage in man: Elliptocytosis and blood groups I, families 1 and 2. Ann. Eugen. 1953, **17:** 267.
28. Young, L. E. and Miller, G.: Differentiation between congenital and acquired forms of hemolytic anemia. Am. J. Med. Sc. 1953, **226:** 664.

THE EFFECT OF HYDROCORTISONE UPON THE COURSE OF PNEUMOCOCCAL PNEUMONIA TREATED WITH PENICILLIN*

HENRY N. WAGNER, JR.†, IVAN L. BENNETT, JR., LOUIS LASAGNA, LEIGHTON E. CLUFF, MIRIAM B. ROSENTHAL‡, AND GEORGE S. MIRICK

The Osler Medical Service of the Johns Hopkins Hospital, the Medical Service of the Baltimore City Hospitals, and the Divisions of Biology and Clinical Pharmacology of the Department of Medicine, Johns Hopkins University School of Medicine

Received for publication October 17, 1955

In a number of experimental situations, it has been shown beyond question that initiation of bacterial or viral infection in animals receiving cortisone or adrenocorticotrophic hormone (ACTH) is followed by unusually rapid progression of the infectious process with the outcome overwhelmingly in favor of the parasite. In man as well, the occurrence of a variety of infections in patients receiving adrenal steroids is well documented although it has not, for obvious reasons, been possible to assay with exactness the possible intensification of the infectious process in man by these hormones. On the other hand, surprisingly few experiments have been reported in which administration of steroids was begun *after* infection had been established in animals or where concomitant antibiotic therapy was employed. Furthermore, enough instances in which corticosteroids have appeared to facilitate recovery in severe human infections by suppressing so-called "toxemia" have now been described to make it clear that cautious exploration of the clinical usefulness of these drugs as adjuvants to specific antimicrobial therapy is worthwhile. The present report describes a study of the effect of hydrocortisone upon the course of pneumococcal pneumonia treated with penicillin, a human infection with a rather predictable clinical course.

METHOD OF STUDY

Patients.—All cases of pneumococcal pneumonia without bacterial complications (such as meningitis or empyema) admitted to the ward services of the Johns Hopkins Hospital and the Baltimore City Hospitals during the period from October 1, 1954 to May 31, 1955 were included in the study. Every patient in whom initial history and examination suggested the diagnosis of pneumococcal infection of the lung was tentatively placed in the experimental group. When bacteriologic confirmation of the diagnosis was not obtained, a patient was subsequently excluded from the study. Twelve of 125 patients suspected of having

*This study was supported in part by Grant A-660 of the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

† Present address: National Heart Institute, Bethesda, Md. (Formerly Assistant Resident Physician, Johns Hopkins Hospital).

‡ Present address: 1410 Willis Street, Richmond, Va. (Formerly Assistant Resident physician, Baltimore City Hospitals).

TABLE I

Comparison of Initial Data on 52 Patients Treated with Hydrocortisone and Penicillin with 61 Controls Treated with Penicillin Alone

	Hydrocortisone		Control		Total	
	No. Pts.	%	No. pts.	%	No. pts.	%
No. Pts.	52	100	61	100	113	100
Male	28	54	48	79	76	67
Female	24	46	13	21	35	33
White	10	19	14	23	24	21
Negro	42	81	47	77	89	79
Under 40 yr.	28	54	31	51	59	52
40–59 yr.	19	38	16	26	35	31
60 yr. or over	5	10	14	23	19	17
Bacteremia	9	17	4	7	13	12
Shock	3	6	0	0	3	3
Multilobar dis.	7	13	11	18	18	16
Alcoholism	15	29	24	39	39	35
Other disease*	10	19	17	28	27	24

* Heart failure, chronic lung disease, diabetes, etc.

TABLE II

Comparison of Patients in Present Study with Those Observed in Recent Years

Period of Study	Patients		Deaths		Bacteremia		Multilobar		Shock	
	No.	%	No.	%	No.	%	No.	%	No.	%
Jan. '50–July '50 (1)	100	100	1	1	22	22	11	11	4	4
July '50–June '51 (2)	116	100	3	2.9	31	26.7	32	27.6	3	2.6
July '51–May '52 (3)	102	100	4	3.9	10	9.8	25	24.5	1	1
Oct. '54–May '55 (Present study)	113	100	2	1.8	13	11.5	18	16	3	4.2

pneumococcal pneumonia were eliminated from consideration because of failure to obtain positive cultures, leaving 113 proven cases as the basis for final analysis. Table I summarizes the information on 52 patients treated with hydrocortisone and penicillin and 61 control patients treated with penicillin alone. It can be seen that the groups were comparable in age, sex, race, and in the incidence of major findings known to indicate severity of infection or poor prognosis. Furthermore, a comparison of mortality, incidence of bacteremia, multilobar involvement, and shock in the 113 patients with those in three groups of patients with pneumococcal pneumonia observed at these institutions in recent years indicated that there was nothing unusual about this "pneumonia season" (Table II) in Baltimore.

Therapy.—Each patient was given aqueous potassium penicillin G* intramuscularly in a dosage of 300,000 units every 12 hours. This was continued for 7 days or until the patient had been afebrile for 48 hours, whichever was shorter. In the case of patients known to be receiving hydrocortisone, an afebrile period of 48 hours after discontinuing the hormone was required before cessation of antibiotic treatment.

* Aqueous penicillin G, hydrocortisone, and placebo tablets were kindly supplied by the Charles Pfizer Company.

In addition, each patient among the first 85 studied whose hospital number ended in an odd integer was given a total of 560 mgm. of hydrocortisone* orally during the 5 days after admission on the following schedule:

80 mgm. on admission.
60 mgm. every 6 hours for 3 doses.
40 mgm. every 6 hours for 4 doses.
20 mgm. every 6 hours for 4 doses.
10 mgm. every 6 hours for 4 doses.
10 mgm. every 12 hours for 2 doses.

The last 28 patients studied received either hydrocortisone or a placebo*, cases being selected at random rather than by history number. Neither the patient nor the physicians managing him knew which therapy he was receiving. As noted in Table I, there were 61 controls and 52 hormone-treated patients.

Bacteriology.—Microscopic examination of sputum stained by Gram's method was carried out immediately after the patient's admission. Before therapy was instituted, sputum and blood cultures were obtained. Sputum was also inoculated intraperitoneally into a mouse. Tail blood and peritoneal washings from the mouse were cultured and tested for the Quellung reaction on the following day. In every instance, pneumococci were typed as the predominant organism from sputum and, as indicated in Table I, pneumococci were isolated from the blood of 13 patients.

Other Laboratory Studies.—The following studies were done at the time of admission: hematocrit, erythrocyte sedimentation rate, total and differential leukocyte count, serum bilirubin, sodium, potassium, and chloride, complete urinalysis, and X-ray of the chest. During the patient's course these were repeated at intervals: weekly hematocrit, urinalysis and sedimentation rate twice weekly, leukocyte count daily until normal, serum bilirubin and electrolytes twice weekly, and X-ray of the chest twice weekly.

Clinical Observations.—Rectal temperature response, time of disappearance of pleuritic pain and changes in physical findings were carefully noted and recorded for all patients. In addition, 45 patients in the series (24 hydrocortisone, 21 controls) were studied specifically in regard to subjective manifestations throughout the course of their disease. A single observer saw all of these patients daily during their illness. A comparison of this subgroup with the entire experimental population revealed that they were similar with respect to age, race, sex, fever and other objective criteria. The method employed in obtaining information from patients was as follows: On the day after admission, each patient was interviewed. The standard opening question was, "How are you feeling today?" If the patient answered "Better" (the usual response), the next question was, "In what way do you feel better?"

The patient was then allowed to speak without prompting until he was apparently finished. If all points of interest were not spontaneously commented upon, the patient was asked specifically whether appetite, pain, and cough were better, worse, or unchanged. Similar questions were asked daily in order to determine when appetite began to improve, pain to lessen, and cough to diminish and also when each had returned to normal. A further specific endpoint was the completely normal or asymptomatic state. Mood changes were noted carefully and if euphoria or dysphoria was evident the patient was questioned on this point. Similarly, patients who appeared to be disturbed or disoriented were further studied. It should be stressed that all patients were seen daily by the same observer and that answers to questions were written down immediately each day, verbatim where possible. It was because the single observer could not always be sure of avoiding bias by remaining ignorant

* See footnote, page 198.

of the patient's medication that the addition of placebo controls was instituted in the latter part of the study.

RESULTS

Mortality.—There were two deaths among the 113 patients, one in the hydrocortisone-treated group and one in the control group.

Case 1: M. H., 70 year white male (JHH 696009). Patient was admitted with fever, cough, and pleuritic chest pain of 5 days duration. He had had several shaking chills. These symptoms had followed acute coryza. He was a chronic alcoholic known to have had delirium tremens on several occasions. *Examination* showed blood pressure of 110/70 and rectal temperature of 103°F. The patient was cyanotic and stuporous and there were signs of consolidation over both lungs, particularly the right. Leukocyte count was 21,000, NPN 107 mgm. per cent, serum bilirubin 2.3 mgm. per cent, and blood cultures grew Type 3 pneumococci. He was given penicillin without hydrocortisone, a tracheotomy was performed to facilitate tracheal toilet, and tetracycline was added to the regimen on the third day. He remained febrile and stuporous and abdominal distention became marked. He died on the fifth day; autopsy was not permitted.

Comment: This elderly alcoholic with multilobar Type 3 pneumococcal pneumonia and bacteremia was treated with antibiotics alone. He was azotemic and icteric and was the only patient in the control group who died.

Case 2: E. B., 85 year white female (BCH 192364). Patient was admitted because of dyspnea of 10 days duration. She had had angina pectoris and chronic congestive heart failure for 15 years. Additional digitalis and an oral mercurial diuretic given 6 days before admission resulted in no improvement. *Examination* showed blood pressure of 130/80 and rectal temperature of 100.2°F. She was emaciated, dyspneic and cyanotic. There was advanced emphysema with many moist rales over the lower lung fields. There was no edema. Leukocyte count was 37,400, and NPN 89 mgm. per cent. The sputum grew Type 19 pneumococci and X-ray of the chest showed generalized pulmonary fibrosis with patchy pneumonic infiltration in the right upper and middle fields. She was given penicillin and hydrocortisone with symptomatic response although she complained of continued weakness and was unable to eat. On the fifth day, when hydrocortisone dosage was reduced to 10 mgm. every 6 hours, she became pale and blood pressure dropped to 72/0. Immediately after injection of 100 mgm. of hydrocortisone intravenously, blood pressure rose to 100/60 and she became alert. She was continued on penicillin, 100 mgm. of hydrocortisone daily and in addition, treatment with streptomycin and isoniazid was instituted because of the possibility of pulmonary tuberculosis. She remained dyspneic, weak, and unable to eat and died quietly on the twenty-eighth hospital day. At autopsy, the lungs showed extensive

emphysema without infection; the adrenals were normal; the only other finding of note was severe coronary arteriosclerosis.

Comment: This 85 year old woman with chronic heart disease and emphysema was the only patient in the group given hydrocortisone who died. She responded symptomatically to hydrocortisone, but expired after a prolonged downhill course. Autopsy revealed no residual infection.

Complications.—Suppurative complications such as empyema, meningitis, pericarditis, etc. did not occur among the patients in either group. Pleural effusion of sufficient extent to warrant aspiration of the chest developed in 4 patients given penicillin alone and in 3 given hydrocortisone; all fluids were sterile and in no case was it necessary to perform thoracentesis more than once.

All blood cultures taken after initiation of therapy were negative and of 39 sputums cultured on the second day of treatment (16 controls, 23 hydrocortisone), none grew pneumococci.

Sixty-seven patients (35 controls, 32 hydrocortisone) were followed with serial roentgenograms of chest until complete *radiographic clearing* took place. Other patients either failed to return to the out-patient department at all or were lost to follow-up before chest X-rays had become completely normal. Resolution was delayed for more than 4 weeks in 4 control and 5 hydrocortisone-treated patients. Clearing finally occurred in all. The longest period required for restoration of normal X-ray of the chest, 61 days, was for one of the control group. In none of these cases was delay in resolution accompanied by symptoms or debility; all 9 patients returned to normal activity within 2 weeks after discharge from the hospital.

Reactions to penicillin were limited to the occurrence of drug fever in one patient among the controls. This was confirmed by readministration of the antibiotic and was unaccompanied by rash, hematologic changes or subjective discomfort.

The common complications of adrenal steroid therapy (4) were not observed. There was no instance of electrolyte imbalance, aggravation of hypertension, or "flare-up" of tuberculosis. Of 9 patients with *diabetes* among the 113, 4 were controls and 5 received hydrocortisone. In no instance was management of this disease unusually difficult. Of interest, in view of the untoward effects of adrenal steroids upon *peptic ulcer*, was the occurrence of massive hematemesis in one patient 10 days after discharge from the hospital for pneumococcal pneumonia. This patient was readmitted and finally underwent subtotal gastric resection. He had received penicillin *without* hydrocortisone for pneumonia!

The possible *psychiatric effects* of hydrocortisone are discussed below.

One patient who was begun on hydrocortisone shortly after admission to the hospital with pneumonia and hypotension developed striking *hypothermia* and was found to have *acute renal failure*. Because this was the only instance in

which it seemed likely that adrenal steroids had exerted an untoward effect, the case is presented in detail.

Case 3: M. K., 40 year Negro female (JHH 700377). Patient was admitted to the hospital with fever and right pleuritic pain. She had been ill with indefinite upper respiratory symptoms for at least a week but chest pain had begun only 2 days before, at which time she had also had a single shaking chill. She had never been hospitalized before and denied serious illness in the past. ***Physical examination*** showed blood pressure of 80/45, respirations 28, and rectal temperature of 101.8°F. She was lethargic and grunted with each respiration, splinting the right chest. There was no evidence of dehydration; there was obvious scleral icterus. The neck was supple. There were signs of consolidation over the lower right lung field posteriorly and laterally. The heart was normal and except for abdominal distention, no other significant findings were described. Leukocyte count was 18,700 and hematocrit 37 per cent; erythrocyte sedimentation rate 51, (Wintrobe uncorrected); stool negative for blood; serum bilirubin 7.5 mgm. per cent, 5.8 mgm. direct. Admission urinalysis (catheter) showed specific gravity 1.014, acid reaction, 0 sugar, 2+ albumin, with 1–2 WBC and occasional RBC per high-power field after centrifugation; there were no casts; the urine contained bile (Harrison). X-ray of the chest

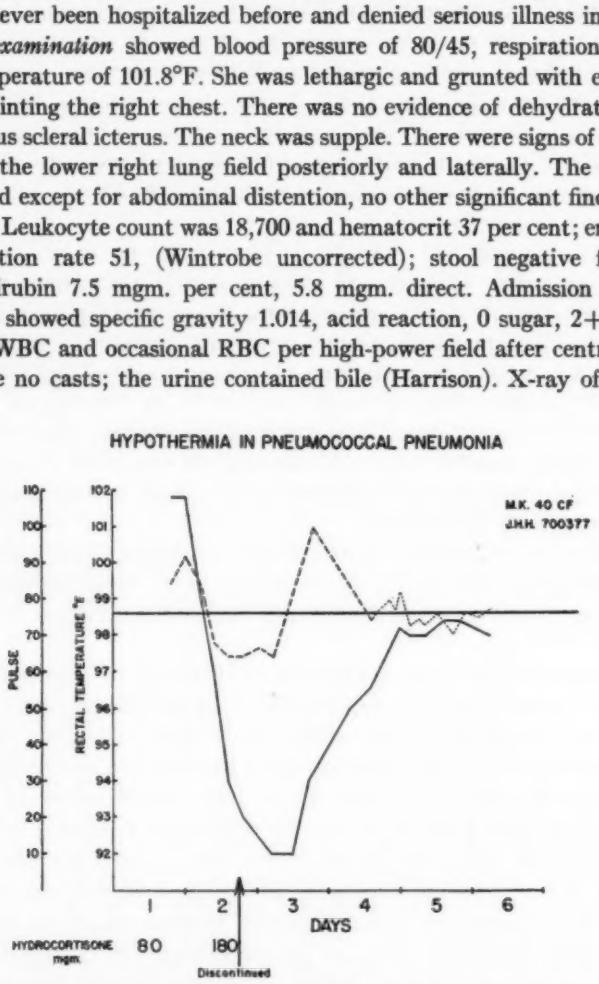


FIG. 1. Temperature course in Case 3. The unbroken line is rectal temperature; the dotted line, the pulse rate. After discontinuance of hydrocortisone, it required nearly 36 hours for the patient's temperature to reach normal levels.

confirmed the presence of consolidation of the entire right lower lobe and cultures of blood and sputum grew Type 1 pneumococci.

She was given 300,000 units of aqueous penicillin intramuscularly every 12 hours and was begun on hydrocortisone, receiving 260 mgm. orally during the first 24 hours of hospitalization. Her subsequent course was characterized by hypothermia, hypotension and progressive renal failure.

Within 12 hours, the patient's rectal temperature had fallen to 94°F. and within 24 hours to 93°F. At this point, hydrocortisone was discontinued and, as is illustrated in Figure 1, the patient's temperature fell to 92° and then slowly climbed to 98° during the next 36 hours. During this episode, she was somewhat lethargic but remained oriented and had no complaints. The patient's blood pressure remained at low levels and it soon became apparent that she was almost anuric. She was given *l*-norepinephrine (Levophed) intravenously for 12 hours with prompt return of blood pressure to normal but no change in urine output. At this time, it was reported that the blood NPN on admission was 200 mgm. per cent. The use of *l*-norepinephrine was discontinued and she was begun on a regimen that balanced intake of fluid and electrolytes against output and "insensible loss." Blood pressure gradually rose until it reached normal levels at the end of the first week and mildly hypertensive levels for several days thereafter. Figure 2 outlines the course of this patient's renal failure. Management was simplified considerably by collection of all vomitus and its readministration through a gastric tube. Diuresis began

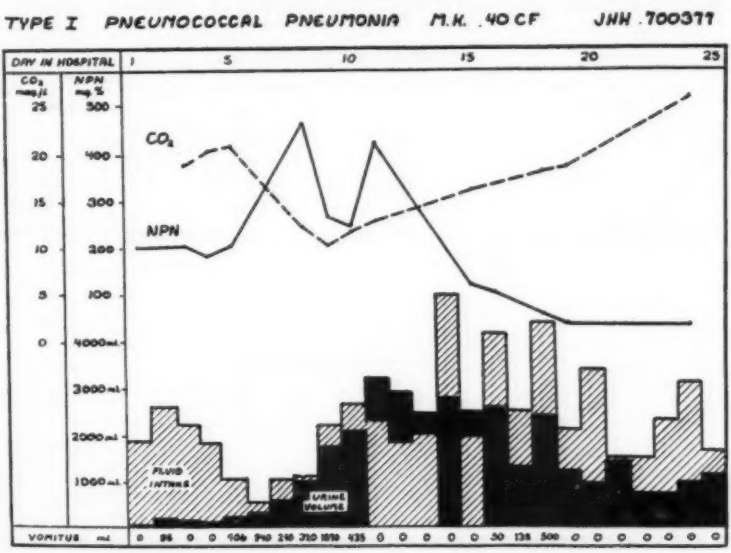


FIG. 2. Diagram of clinical course of renal failure in Case 3.

during the second week after the NPN had reached a high of 468 mgm. per cent. There was never any serious imbalance of electrolytes and, by the twenty-fourth day, NPN was 40 mgm. per cent. She was discharged after 34 days of hospitalization. The bilirubinemia, which had been accompanied by BSP retention of 31 per cent in 45 minutes, cleared rapidly and liver function was normal at the time of discharge. Pneumonia cleared promptly; X-ray of the chest was normal by the third week. She was last seen 4 months after leaving the hospital at which time she was working 3 days a week without complaints. Blood pressure, NPN and urinalysis were normal. Phenolsulfonphthalein excretion was 45 per cent in 2 hours.

Comment: This 40 year old woman was severely ill with Type 1 pneumococcal pneumonia and bacteremia. She was icteric, hypotensive and uremic when admitted. The acute renal failure, presumed to have resulted from prolonged hypotension accompanying the infection, responded to conservative management. The infection in the lung was readily controlled with penicillin. Administration of hydrocortisone was followed by profound hypothermia. While it seems certain that this fall in temperature was attributable to the administration of adrenal steroid similar to the case reported by Kass (5), there is nothing to indicate that the renal failure was related to or influenced by hydrocortisone.

Laboratory findings: No significant differences in laboratory findings were observed in the two groups. Electrolytes, serum bilirubin, urinary findings, leukocyte counts and the rates at which counts returned to normal, and even erythrocyte sedimentation rates were uninfluenced by hydrocortisone.

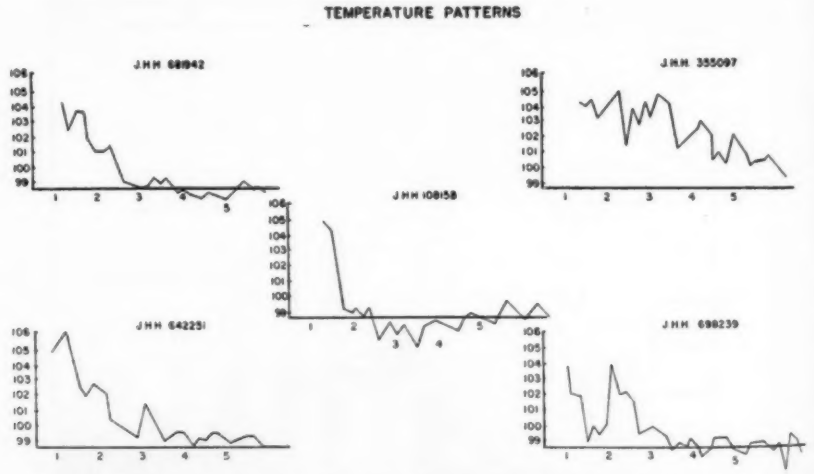


FIG. 3. Examples of temperature course of pneumococcal pneumonia treated with penicillin alone.

Some of the laboratory findings in the entire group of 113 patients are worth noting. Twelve had admission leukocytosis of more than 30,000, in 20 the leukocyte count was less than 10,000 before treatment. Only 2 of the 113 patients had normal erythrocyte sedimentation rates on admission. Fifty-five patients had elevated serum bilirubin (greater than 1.0 mgm. per cent) when first seen; elevation persisted for more than 7 days in only 3 instances and only in the control patient who died (Case 1) did icterus fail to subside completely.

Fever.—In view of the well-known antipyretic action of adrenal steroids (6–10), it is not surprising that the most obvious effect of hydrocortisone in this study was alteration in febrile response.

The disappearance of fever after administration of penicillin in 40 of the *control* patients was prompt with fall by crisis to normal levels (i.e. 99.6°F) within 24–36 hours, but there were many variations in response, some examples of which are shown in Figure 3. Twenty-one of the control patients defervesced by lysis over a period of 5 to 14 days; Figure 4 illustrates this type of "delayed" response. Eleven patients given penicillin alone had brief "secondary" rises in temperature on the day after they had become afebrile by crisis.

With 2 exceptions, the 52 patients given *hydrocortisone* became afebrile by crisis within 24 hours. Characteristically, temperatures fell sharply to slightly subnormal levels and as the dosage of hydrocortisone was decreased, rose toward the normal baseline. The temperature curves of the first 15 patients in the

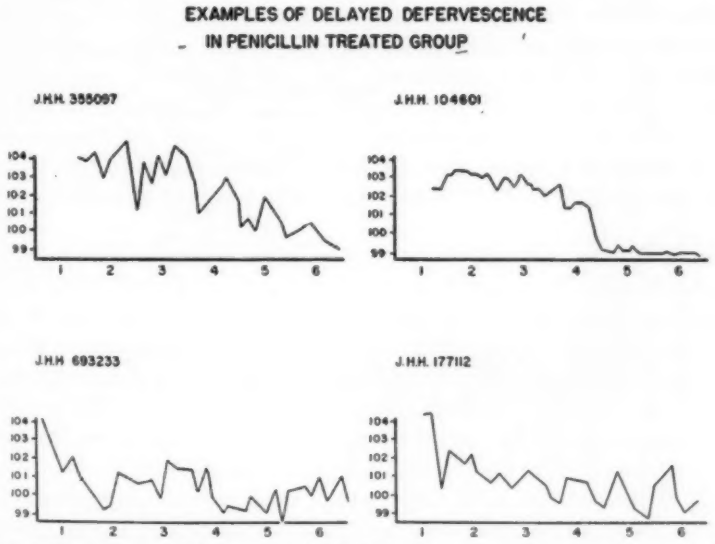


FIG. 4. Examples of defervescence by lysis in pneumococcal pneumonia treated with penicillin alone. Twenty-one of the 61 control patients showed this type of response.

control group are shown in Figure 5 and those of the first 15 patients given hydrocortisone, in Figure 6. Fourteen of the 52 patients given hydrocortisone experienced a recurrence of fever for 24–36 hours after steroid therapy was stopped. This was occasionally accompanied by return of symptoms as mentioned in a later section. This "rebound" is seen in 4 of the records shown in

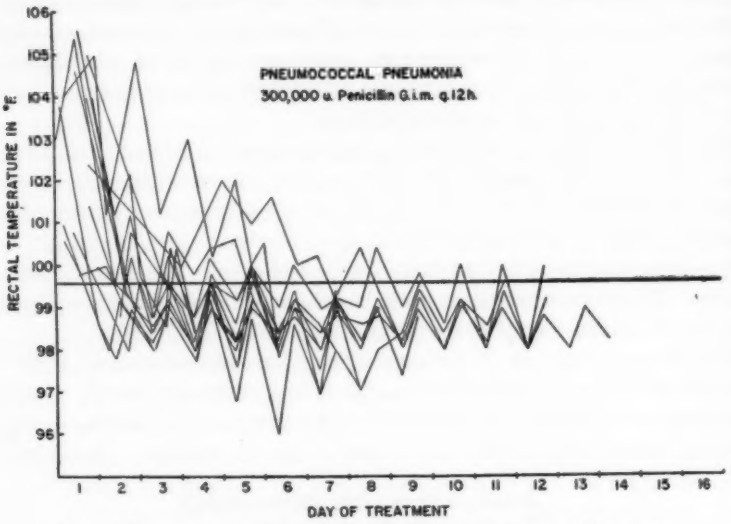


FIG. 5. Temperature course of first 15 patients given penicillin alone.

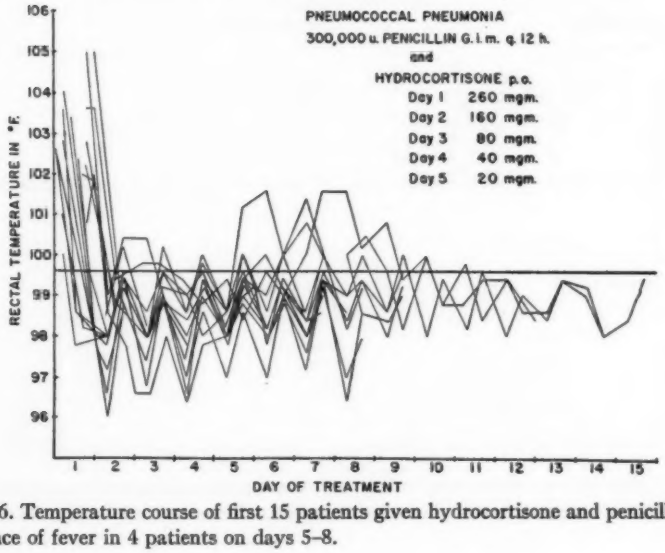


FIG. 6. Temperature course of first 15 patients given hydrocortisone and penicillin. Note recurrence of fever in 4 patients on days 5–8.

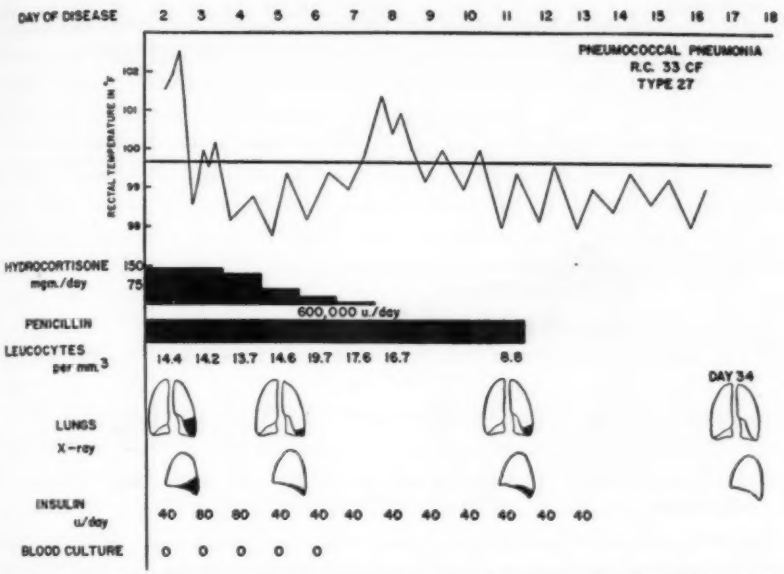


FIG. 7. An example of recurrence of fever as hydrocortisone was discontinued in a patient with diabetes mellitus and Type 27 pneumonia.

Figure 6 and is further illustrated by Figures 7 and 8. In 3 patients return of fever after stopping hydrocortisone was accompanied by apparent increase in X-ray evidence of consolidation; this was definite only in the case shown in Figure 8, was unaccompanied by subjective complaints and in every instance, cleared completely without residual. It was thought that the increased opacification might represent pleural effusion but this was not verified; fluoroscopic findings were equivocal and, in the case shown in Figure 8, thoracentesis yielded no fluid.

Although the transient recurrence of fever after termination of steroid therapy might represent a true "rebound", it seems more likely that fever in most of these patients would have responded to penicillin alone by lysis and that the so-called "recurrence" was, in reality, the end of what would have been a progressively decreasing fever curve had not the major portion been masked by the antipyretic action of hydrocortisone.

Subjective response.—The day to day evaluation of subjective response in 45 patients (21 controls, 24 hydrocortisone-treated) showed, in general, a more rapid *improvement* in symptoms among those given adrenal steroid therapy although much less difference was apparent between the two groups when the times required for complete disappearance of symptomatic complaints were compared. The results are summarized below.

(a) *Appetite.*—All but 3 patients given hydrocortisone reported *increase*

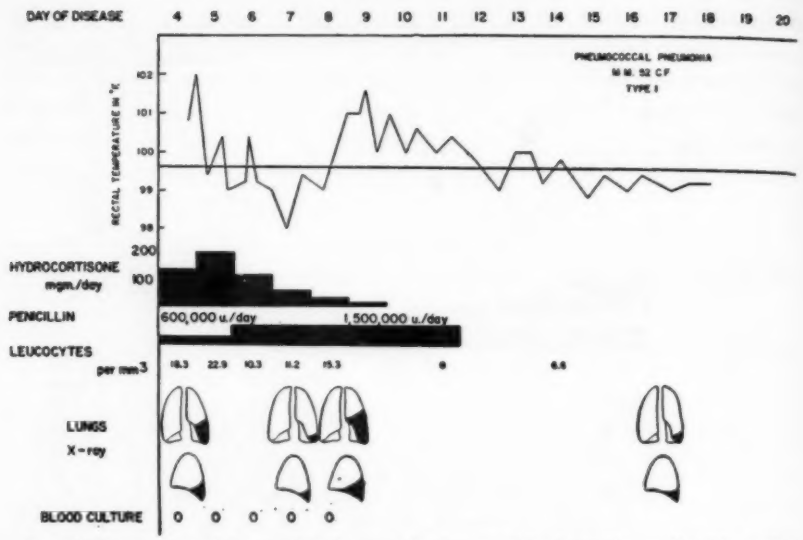


FIG. 8. Recurrence of fever with decrease in dosage of hydrocortisone. In this patient, the X-ray suggested increase in consolidation of the lung although this may have been the result of an effusion. Penicillin was increased to 1,500,000 units daily because of a misunderstanding.

in appetite within 24 hours after initiation of therapy. Two required 48 hours and in one case, 6 days elapsed before improvement was definite. Among the controls, renewed interest in food appeared within 24 hours in 11 patients, 48 hours in 5, 72 hours in 2, and 6 days in 3. There was wide variation in the intervals for *complete restoration* of appetite in both groups, the maximum being 16 days in a hormone-treated patient and 20 in a control. The medians for the groups were: hydrocortisone, 3 days; controls, 4 days.

(b) *Pain.*—All 24 patients given hydrocortisone reported *decrease* in chest pain within 24 hours as did 18 of 21 controls. Relief was noted by 2 control patients at 48 hours and by 1 on the fourth day. *Complete subsidence* of pleuritic pain required as long as 9 days in one patient given steroids and one control patient continued to have recurrences of pain until the nineteenth day. The median times for complete relief were: hydrocortisone group, 2 days; controls, 4 days.

(c) *Cough.*—*Decrease* in cough occurred within 24 hours in all patients given hydrocortisone. Among the controls, 16 patients reported improvement at 24 hours, 3 at 48 hours, and 2 noted decrease in this symptom only after 4 days. The *disappearance* of cough (or its return to a "normal" level) required 1 to 13 days for hydrocortisone-treated patients and 2 to 20 days for the controls. The median for each group was 6 days.

(d) *Asymptomatic state.*—The lengths of time elapsing between initiation of treatment and the patient's reporting that he was "as good as new" showed a wide spread; the ranges were 1 to 16 days for hydrocortisone and 4 to 21 days for controls with a 6 day median in each group.

(e) *Return of symptoms.*—In much the same manner that fever returned briefly in several patients as the dosage of hydrocortisone was reduced, 5 patients in the hormone treated group experienced recurrences of chest pain, 2 on the third day, 2 on the fourth, and 1 on the seventh. Two control patients experienced transient pleuritic pain after initial subsidence, 1 on the fifth day, and 1 on the seventh.

Return of anorexia after initial improvement in appetite accompanied a mild depressive reaction in 1 patient given hydrocortisone (see following section).

Hydrocortisone, in the dosage employed in this study seemed to act as an analgesic and antipyretic drug with some antitussive and appetite-stimulating effects. The few instances of return of discomfort as adrenal steroid dosage was reduced are most easily interpreted as unmasking of symptoms that the drug had suppressed. It is possible that continuation of hydrocortisone beyond the period arbitrarily selected for study would have prevented these "recurrences."

Disturbances of Mood and Orientation.—Excluding a number of patients who were confused or disoriented on admission but quickly returned to normal, there were 3 patients in each group who manifested significant psychiatric abnormalities during the course of therapy.

(a) *Hydrocortisone-treated group.*—The first patient, a 43 year old Negro male, denied alcoholic intake other than 3–4 bottles of beer daily. He experienced rapid improvement in symptoms on penicillin and hydrocortisone. On the fifth night in the hospital, however, he imagined he saw ghostly dancing figures, became frightened because "someone was trying to kill him", and was grossly disoriented as to time and place. He received his last dose of steroid 12 hours after onset of these symptoms and within 3 days, it was no longer possible to detect any behavioral abnormalities. A psychiatrist classified the episode as a delirious reaction and was unable to detect any evidence of pre-existing psychiatric abnormality.

The second patient, a 22 year old Negro female, was much improved within 24 hours after initiation of treatment with penicillin and hydrocortisone. On the following day, however, she became sullen and depressed, attributing this change in mood to "not being visited by someone I was expecting." She ignored questions or answered curtly, had repeated spells of weeping, and when queried about the reasons for her actions answered, "I just like to be alone sometimes," or "I'll feel blue until I go home." Her appetite lagged until hydrocortisone was discontinued at which time she brightened, seemed happy, and manifested

renewed interest in food. A psychiatrist felt that the episode was a mild depressive reaction to her illness and separation from her child.

The third patient was a 42 year old Negro male, known to be a chronic alcoholic. He responded well to hydrocortisone and penicillin insofar as fever, etc. were concerned but continued to look unhappy, tense, and anxious and had gross tremors of the hands for several days. He refused to admit discomfort, saying there was "nothing to be happy or unhappy about."

(b) *Controls.*—Among the controls, the first patient, a 69 year old Negro male, and a heavy consumer of alcohol, had visual hallucinations when admitted and on the fourth day had to be restrained because of violent complaints that other patients had stolen his keys. He became asymptomatic after several days.

The second patient, a 33 year old white male, also known to be a severe alcoholic, had visual and auditory hallucinations for 1 week after admission and subsequently required restraint because of paranoid delusions involving patients and ward personnel. He cleared completely within 3 weeks and it was learned from the family and the records of another hospital that similar episodes had followed abstinence from alcohol in the past.

The third patient, a 47 year old Negro, also an alcoholic, responded well to penicillin but on the fifth day became suddenly disoriented and left the hospital in scanty attire to be found several blocks away some hours later. He was given intramuscular reserpine and within 24 hours was oriented and cooperative. The remainder of his hospital stay was uneventful.

DISCUSSION

In the present study, administration of hydrocortisone in decreasing doses for 5 days to patients receiving penicillin for pneumococcal pneumonia resulted in quicker symptomatic improvement and greater comfort during the acute phase of the disease than were observed in patients given penicillin alone. In contrast, there was essentially no shortening of the time required for *complete* restoration of well-being and return to "normal." As was expected, the most obvious objective difference between the two groups was the more rapid disappearance of fever in patients given the hormone. Discontinuance of hydrocortisone was followed in several cases by brief recurrence of fever and in a few by return of chest pain or other discomfort. The arbitrarily chosen schedule of 5 days for administration of hydrocortisone in diminishing dosage probably led to unmasking of these manifestations after initial suppression. It seems altogether probable that continuation of the hormone for a few days longer would have eliminated these "recurrences" or "rebounds" and that significant acceleration of return to "normal well-being" could have been achieved.

It is clear that there was no worsening of infection by hydrocortisone and the incidence of complications of steroid administration was negligible. The patient who bled massively from a peptic ulcer shortly after treatment with

penicillin alone would undoubtedly have been pointed out as a bad result of hormone therapy had he happened to fall into the other study group. The only disturbing effect of corticosteroid treatment was a single instance of profound hypothermia, similar to that reported by Kass (5) in a patient given both cortisone and aspirin. There were no serious consequences.

A detailed consideration of the vast accumulation of experimental studies of adrenal steroids and infection is beyond the scope of this report. Several excellent reviews are available (11–13). Certain comments, however, seem pertinent. The many unequivocal demonstrations that initiation of infection in experimental animals treated with corticosteroids is followed by acceleration of the disease process and increased mortality cannot be taken wholesale and uncritically as supporting the sweeping conclusion that these hormones are contraindicated in human infections. Disregarding entirely the critical matter of dosage and such complexities as variations in adrenal cortical secretion among species or even different strains of the same species (14–17), several aspects of the management of human infections can be considered:

(a) Patients ordinarily present themselves for treatment *after* infection has been established and illness has occurred. It is entirely possible that the effects of corticosteroids administered at such a time may be quite different from those of ***premedication*** with hormone, the usual experimental procedure (there are a few exceptions, but this point has received surprisingly little attention in laboratory studies).

(b) It is also obvious that therapy of human infections is not ordinarily or necessarily limited to adrenal steroids; the proper antibiotic or other specific chemotherapeutic agent is given not once, but repeatedly, and not in minimal curative doses, but in amounts that usually exceed this level many fold. In a series of critically conducted experiments, Jawetz (18) found that prior administration of cortisone interferes with the curative action of antibiotics in bacterial infections of mice. However, this investigator also went on to show that the simple procedure of increasing the dosage of the antibiotic entirely abolished this effect of cortisone. This, of course, corresponds to the usual procedure in giving antibiotics to human patients, where deliberate overtreatment is the rule. For example, the demonstration by Tillett (19) that as little as 40 to 100 thousand units of penicillin daily suffices to control pneumococcal pneumonia has by no means resulted in the universal adoption of this as the optimum dosage of the antibiotic for this disease.

(c) Furthermore, there are now several reports indicating that the administration of corticosteroids to patients with various types of infection, usually in conjunction with antibiotic or other specific treatment, is not only unattended by disastrous complications, but may be beneficial. Studies by Woodward and his co-workers who gave cortisone alone to patients with typhoid (20), by Smadel, Ley, and Diercks (21), and by Wisseman, *et al.* (22),

have shown conclusively that cortisone given during the early stages of typhoid treated with chloroamphenicol produces a much more striking clinical response than does chloramphenicol alone. Similarly, the "febrile-toxemic" state has been relieved dramatically by supplementing antibiotic therapy with cortisone in Rocky Mountain Spotted Fever (23) and also in brucellosis (24). The addition of oral cortisone to the usual regimen of penicillin and antiserum reduced mortality from 85 per cent to 40 per cent in a series of 40 consecutive cases of severe tetanus studied under controlled conditions by Lewis and his collaborators (25). While the ability of corticosteroids to aggravate tuberculous infection in animals and in man is well established (26–30), there is much to indicate that cortisone combined with specific antituberculous drugs exerts a beneficial effect in this disease (31–33), especially in tuberculous meningitis (34). Kinsell, et al. (35–37) have employed adrenal steroids as adjuvants to chemotherapy in a large number of infections, many of them in surgical patients, with good results and a low incidence of serious or troublesome complications attributable to the hormones.

(d) To return to pneumococcal pneumonia, the excellent results achieved with penicillin alone and the equal effectiveness of other antibiotics in this infection (1, 3, 38) have reduced mortality in the disease to a point that it can hardly be regarded as a major therapeutic problem at present. In the study reported here, the incidence of such complications as shock was too small to allow evaluation of hydrocortisone's effect on mortality. There is no basis for advocating the use of cortisone and related compounds as the sole agent in treatment of bacterial infections. Shortly after ACTH became available, Kass, Ingbar, and Finland (39) described 3 cases of pneumococcal pneumonia treated with this drug alone. In all cases there was striking defervescence, but bacteremia persisted in one, one patient continued to produce rusty sputum, and the third developed empyema. It is difficult to state definitely that ACTH was deleterious in these patients; that the infections would have behaved differently had penicillin been given instead of ACTH is definite enough, but their course if allowed to remain untreated with either agent can only be speculated upon. Certainly, persistent bacteremia, continued sputum production and the development of empyema were not uncommon in the days before specific therapy was available for pneumococcal infections. The definite symptomatic benefit of hydrocortisone in pneumonia and the absence of significant harmful effects in the present study justifies further exploration of these hormones as adjuncts to antibiotics and chemotherapeutic agents in other human infections in which mortality is more of a problem than it is in lobar pneumonia.

(e) Finally, it may be pointed out that *symptomatic relief*, usually disregarded in favor of more objective criteria in animal experimentation, is far from being a minor consideration in the management of human disease. Indeed, infections that threaten life are far outnumbered in clinical practice by a host of self-

limited febrile illnesses for many of which specific treatment is lacking. If it becomes possible to relieve the prostration and malaise accompanying these diseases without exposing the patient to the risk of serious complications, the physician's job and the patient's lot will have been made easier. To this end, a controlled study of the effect of aspirin upon the symptoms of pneumococcal pneumonia treated with penicillin is now under way in this clinic.

SUMMARY AND CONCLUSIONS

The effect of administration of hydrocortisone orally for 5 days upon the course of pneumococcal pneumonia was studied in 52 patients and compared with a control group of 61 patients treated with penicillin alone. More rapid defervescence and improvement in symptoms were noted in the hormone-treated patients; manifestations of disease occasionally recurred when hydrocortisone was withdrawn. There were two deaths, one in each study group. The only disturbing reaction attributable to corticosteroid therapy was severe hypothermia in one patient necessitating withdrawal of the drug. The results are discussed in relation to other reports of the use of adrenal hormones in human infections. It is concluded that the symptomatic benefit of hydrocortisone and the absence of any evidence to indicate aggravation of the infectious process in pneumococcal pneumonia justifies further cautious exploration of adrenal steroids as adjuvants to specific antimicrobial therapy.

BIBLIOGRAPHY

1. Austrian, R., Mirick, G. S., Rogers, D. E., Sessoms, S. M., Tumulty, P. A., Vickers, W. H., Jr. and Zubrod, C. G.: The efficacy of modified oral penicillin therapy of pneumococcal lobar pneumonia. Bull. Johns Hopkins Hosp., 1951, **88:** 270.
2. Austrian, R., Barondess, J., Mirick, G. S., Sessoms, S., Sweeney, J. E. and Zubrod, C. G.: The efficacy of aureomycin in the treatment of pneumococcal lobar pneumonia. Bull. Johns Hopkins Hosp., 1951, **89:** 407.
3. Austrian, R., Cluff, L. E., Mirick, G. S., Sessoms, S. M. and Zubrod, C. G.: A comparison of the efficacy of chloramphenicol (Chloromycetin) and of penicillin in the treatment of pneumococcal lobar pneumonia. Bull. Johns Hopkins Hosp., 1952, **91:** 323.
4. Thorn, G. W., Jenkins, D., Laidlaw, J. C., Goetz, F. C., Dingman, J. F., Arons, W. L., Streeten, D. H. P. and McCracken, B. H.: Pharmacologic aspects of the adrenocortical steroids and ACTH in man. New England J. Med., 1953, **248:** 232, 284, 323, 369, 414, 632.
5. Kass, G. H.: Hypothermia following cortisone administration. Am. J. Med., 1955, **18:** 146.
6. Kass, E. H. and Finland, M.: Effect of ACTH on induced fever. New England J. Med., 1950, **243:** 693.
7. Recant, L., Ott, W. H. and Fischel, E. E.: The antipyretic effect of cortisone. Proc. Soc. Exp. Biol. and Med., 1950, **75:** 264.
8. Duffy, B. L., Jr. and Morgan, H. R.: ACTH and cortisone aggravation or suppression of the febrile response of rabbits to bacterial endotoxin. Proc. Soc. Exp. Biol. and Med., 1951, **78:** 687.

9. Douglas, W. W. and Paton, W. D. M.: The hypothermic and antipyretic effect of preparations of ACTH. Lancet, 1952, **1:** 342.
10. Bennett, I. L., Jr. and Beeson, P. B.: The effect of cortisone upon reactions of rabbits to bacterial endotoxins with particular reference to acquired resistance. Bull. Johns Hopins Hosp., 1953, **93:** 290.
11. Kass, E. H. and Finland, M.: Adrenocortical hormones in infection and immunity. Ann. Rev. Microbiol., 1953, **7:** 361.
12. Shwartzman, G.: *The Effect of ACTH and Cortisone Upon Infection and Resistance.* Columbia University Press, New York, N. Y., 1953.
13. Thomas, L.: Infectious diseases; effects of cortisone and adrenocroticotrophic hormone on infection. Ann. Rev. Med., 1952, **3:** 1.
14. Bush, I. E.: Paper chromatographic study of the secretion of the adrenal cortex in various mammalian species. J. Physiol., 1951, **115:** 12 (Proc. Physiol. Soc., May 19, 1951).
15. Bush, I. E.: Species differences in adrenocrotical secretion. J. Endocrinol., 1953, **9:** 95.
16. Kass, E. H., Hechter, O., Macchi, I. A. and Mou, T. W.: Changes in patterns of secretion of corticosteroids in rabbits after prolonged treatment with ACTH. Proc. Soc. Exp. Biol. and Med., 1954, **85:** 583.
17. Kass, E. H., Hechter, O., Mou, T. W. and Lune, M. B.: Effects of adrenal steroids on resistance to infection. A.M.A. Arch. Int. Med., 1955, **96:** 397.
18. Jawetz, E. and Merrill, E. R.: The effect of cortisone upon the therapeutic efficacy of antibiotics. Science, 1953, **118:** 549.
19. Tillett, W. S., McCormack, J. E. and Cambier, M. J.: The treatment of lobar pneumonia with penicillin. J. Clin. Inv., 1945, **24:** 589.
20. Woodward, T. E., Hall, H. E., Diaz-Rivera, R., Hightower, J. A., Martinez, E. and Parker, R. T.: Treatment of typhoid fever. II. Control of clinical manifestations with cortisone. Ann. Int. Med., 1951, **34:** 10.
21. Smadel, J. E., Ley, H. L. and Diercks, F. H.: Treatment of typhoid fever. II. Combined therapy with cortisone and chloramphenicol. Ann. Int. Med., 1951, **34:** 1.
22. Wisseman, C. L., Jr., Paterson, P. Y., Smadel, J. E., Diercks, F. H. and Ley, H. L., Jr.: Studies on cortisone and antibiotics for prompt therapeutic control of typhoid fever and scrub-typhus. J. Clin. Inv., 1954, **33:** 264.
23. Workman, J. B., Hightower, J. A., Borges, F. J., Furman, J. E. and Parker, R. T.: Cortisone as an adjunct to chloramphenicol in the treatment of Rocky Mountain spotted fever. New England J. Med., 1952, **246:** 962.
24. Magill, G. B., Killough, J. H. and Said, S.: Cortisone and combined antibiotic therapy of acute brucellosis melitensis. Am. J. Med., 1954, **16:** 810.
25. Lewis, R. A., Satoskar, R. S., Joag, G. G., Dave, B. T. and Patel, J. C.: Cortisone and hydrocortisone given parenterally and orally in severe tetanus. J. Am. Med. Assn., 1954, **156:** 479.
26. Lurie, M. B., Zappasodi, P., Dannenberg, A. M., Jr. and Cardona-Lynch, E.: The effect of cortisone and ACTH on the pathogenesis of tuberculosis. Ann. New York Acad. Sc., 1953, **56:** 779.
27. American Trudeau Society: Statements prepared by the Committee on Therapy. Am. Rev. Tuberc., 1952, **66:** 254, 257.
28. Walker, B.: Miliary tuberculosis in a case of acute disseminated lupus erythematosus treated with ACTH. Brit. Med. J., 1952, **2:** 1076.
29. Traut, E. F. and Ellman, J.: Exacerbation of tuberculosis during treatment with cortisone. J. Am. Med. Assn., 1952, **149:** 1214.

30. Capon, A. W.: ACTH, cortisone and tuberculosis. Canad. Med. Assn. J., 1952, **67:** 46.
31. Houghton, L. E.: Combined corticotropin therapy and chemotherapy in pulmonary tuberculosis. Lancet, 1954, **1:** 595.
32. Browne, J. S. L., Aronovitch, M., Beck, J. C., Leith, W. and Meakins, J. F.: The treatment of coexisting Addison's disease and active pulmonary tuberculosis. Am. J. Med. Sc., 1954, **228:** 491.
33. Cochran, J. B.: Cortisone in the treatment of pulmonary tuberculosis. Edinburgh Med. J., 1954, **61:** 238.
34. Ashby, M. and Grant, H.: Tuberculous meningitis treated with cortisone. Lancet, 1955, **1:** 65.
35. Jahn, J. P., Boling, L., Meagher, T. R., Peterson, H. H., Thomas, G., Fisher, B. M., Thill, A. E., Leovy, W. A., Balch, H. E. and Kinsell, L. W.: The combination of ACTH-cortisone-hydrocortisone with antibiotics in the management of overwhelmingly severe infections. Theory and practice based on three years' experience. J. Pediat., 1954, **44:** 640.
36. Kinsell, L. W. and Jahn, J. P.: The use of corticords in association with antibiotics in the management of unusually severe infections. Ann. New York Acad. Sc., 1955, **61:** 397.
37. Kinsell, L. W. and Jahn, J. P.: Combined hormonal-antibiotic therapy in patients with fulminating infections. A. M. A. Arch. Int. Med., 1955, **96:** 418.
38. Kirby, W. M. M., Michel, J. C., Coleman, D. H., Haviland, J. W. and Sparkman, D. R.: Comparison of aureomycin and chloramphenicol in treatment of bacterial pneumonia. J. Am. Med. Assn., 1951, **147:** 110.
39. Kass, E. H., Ingbar, S. H. and Finland, M.: Effects of ACTH in pneumonia. Clinical, bacteriological and serological studies. Ann. Int. Med., 1950, **33:** 1081.

STUDIES ON THE PATHOGENESIS OF FEVER. V. THE FEVER ACCOMPANYING PNEUMOCOCCAL INFECTION IN THE RABBIT*

IVAN L. BENNETT, JR.

Biological Division, Department of Medicine, Johns Hopkins University School of Medicine

Received for publication November 15, 1955

In previous studies (1, 2) it was shown that heat-labile fever-producing substances are present in extracts of rabbit polymorphonuclear leukocytes and in the cell-free fluid of sterile pleural or peritoneal exudates produced by infusion of 0.85 per cent NaCl solution. Tests of many other tissues and cells of the rabbit failed to reveal pyrogenic properties (1, 3). In experiments designed to assess the possible role of these endogenous pyrogenic substances in the pathogenesis of fever, it was found that saline-induced peritonitis in rabbits is unaccompanied by fever and extensive testing of blood and of thoracic duct lymph gave no indication that fever-producing material is absorbed from the peritoneal cavity in significant amounts (4). Investigations of this type have now been extended and the present report describes the finding of heat-labile pyrogenic material at the inflammatory site in rabbits with pneumococcal infections. Evidence is also presented that systemic absorption of this substance occurs and that this absorption can account for the fever accompanying this infection in the rabbit.

MATERIALS AND METHODS

Animals.—Male rabbits of mixed breed and color weighing 2 to 3 kilos were used in all experiments. They were caged in air-conditioned rooms and were fed Rider's Laboratory Pellets (without antibiotics) *ad libitum*. No supplementary vegetables were given.

Infections.—The SV-1 strain of *Diplococcus pneumoniae*, Type I was obtained from Dr. Colin McLeod. The organism was plated on blood agar, its type was confirmed with specific antiserum, and a smooth colony was inoculated into a flask containing 25 ml. of tryptose phosphate broth to which had been added 2.5 ml. of defibrinated rabbit blood. After incubation at 37°C. for 8 hours, 0.2 ml. quantities of the broth culture were distributed into Wassermann tubes containing 0.5 ml. of defibrinated rabbit blood. These tubes were immersed in a dry-ice and alcohol bath for 30–60 seconds and stored at −70°C. to serve as seed cultures for all subsequent studies. To prepare a culture for infection of animals, the contents of one tube was thawed and added to 10 ml. of tryptose phosphate broth. After incubation at 37°C. for 8 hours, the broth culture was diluted with 0.85 per cent NaCl solution (hereafter referred to as "physiologic saline"). The dose of organisms injected intradermally or intraperitoneally was 0.1 ml. of a 1:10 dilution. Each culture used as an inoculum was checked for purity by plating on blood agar.

Before intraperitoneal injection, animals were shaved on the abdomen. Both flanks were shaved in animals for induction of dermal infections; intradermal injection of organisms

* This study was supported by Grant A-660 of the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

was made on the left, the right side serving as a control area. Peritonitis was invariably fatal within 36 hours if allowed to run its course without administration of penicillin. Spontaneous recovery was observed in only one of 37 animals with dermal infections. The evolution of the skin lesions conformed entirely to Goodner's original description (5).

Collection of Specimens for Fever Tests.—At various intervals after induction of pneumococcal infection, specimens of blood, peritoneal exudate, or thoracic duct lymph were taken to be tested for the presence of pyrogenic material by intravenous injection into normal rabbits. In addition, saline extracts of skin lesions were prepared and tested for the presence of fever-producing material.

(a) *Blood*—Animals were bled by cardiac puncture using 18 gauge needles and 50 ml. syringes. Heparin or 3.5 per cent sodium citrate solution was used to prevent clotting or serum was collected after clotting had taken place at room temperature. Potassium penicillin G was added to all blood and serum in the amount of 10 units/ml. and all specimens were shown to be sterile by culture on blood agar at 37°C. and in thioglycollate broth at room temperature and at 37°C. before testing in normal rabbits.

(b) *Peritoneal Exudates*—Animals with peritonitis were sacrificed by injection of nembutal and the abdomen was opened through a midline incision. Physiologic saline in the amount of 50 or 100 ml. was poured into the peritoneal cavity and, after gentle massage and agitation for 2–3 minutes, as much fluid as possible was removed with 10.0 ml. serological pipettes. After inoculation of a sample into thioglycollate broth, fluids were heated at 60°C. for 30 minutes to kill pneumococci and stored at 4°C. until tested. Smears of these exudates invariably showed myriads of pneumococci and large numbers of polymorphonuclear leukocytes.

(c) *Lymph*—In several animals with pneumococcal peritonitis, thoracic duct lymph was collected for a period of 30–60 minutes immediately before the abdomen was opened to obtain the peritoneal exudate. The method of collection of lymph was similar to that used by Schulz *et al.* (6), except that a polyethylene catheter was used in place of a glass cannula. The technique has been described in detail in a previous report (4). Samples of all specimens were cultured in thioglycollate broth and, after heating at 60°C. for 30 minutes, or addition of 10 units of potassium penicillin G/ml., lymph was stored at 4°C. until tested. Despite the presence of large amounts of purulent exudate in the abdominal cavity and the finding of pneumococci with great regularity in smears and cultures of lymph, the cellular content of the thoracic duct lymph, normally more than 99 per cent lymphocytes, remained virtually unchanged throughout the course of the infection. Only on rare occasions were polymorphonuclear cells observed in lymph specimens. Histologic sections of the lower mediastinal lymph nodes in several animals with pneumococcal peritonitis revealed acute, purulent lymphadenitis with an abundance of polymorphonuclear leukocytes and pneumococci in the nodal sinuses. In view of the observations of Smith and Wood (7, 8) on the pathogenesis of acute bacterial lymphadenitis, this localized accumulation of polymorphonuclear cells and paucity of these cells in efferent lymph were not unexpected findings.

(d) *Extracts of skin*—At various intervals after induction of dermal infection, animals were sacrificed by injection of nembutal and the entire inflammatory lesion was excised from the left flank. The epidermis was carefully removed with a razor-blade and the remaining tissue was ground mechanically in a porcelain mortar with alundum and 10–20 ml. of physiologic saline. Grinding was carried out for 20–30 minutes until a smooth paste was obtained. Enough physiologic saline was added to make a 20 per cent suspension by volume and after the mixture had been centrifuged at 2500 R.P.M. for 20 minutes, the slightly bloody supernatant fluid was decanted for testing. These extracts were heated at 60°C. for

30 minutes and stored at 4°C. until tested. Samples of normal skin (from the right flank) were extracted in similar fashion and used in control tests.

Recording of Temperatures.—Animals with pneumococcal infections were left in their cages and rectal temperatures were taken every 2–8 hours using ordinary clinical thermometers.

To test for the presence of pyrogenic material in exudates, etc., normal rabbits were placed in metal stalls, secured by loose-fitting collars, and rectal temperatures were measured at intervals of 15–30 minutes by means of resistance coils placed in the rectum and an automatic recording device (the Foxboro Rabbit Scanner and Fever Recorder). Temperatures were recorded for at least one hour to obtain a baseline before test-material was injected into a marginal ear vein. All animals had been allowed to become acclimatized to the stalls for several days before being used for actual tests.

The several materials tested for the presence of fever-producing substances were handled as follows:

(a) *Blood*—Heparinized or citrated bloods collected from individual animals were not pooled. Each specimen was divided into two equal parts and these were tested by injection into two normal animals. The smallest single test dose was 21 ml.; the largest, 40 ml. Similarly, unpooled serums were divided and tested in two animals, the volumes used ranging from 17 to 30 ml.

(b) *Peritoneal exudates*—Each exudate was tested by injection of 5.0 ml. amounts into two normal rabbits. If no fevers resulted, 20 ml. of the exudate was injected into a third animal. Only if this larger dose produced no fever was the exudate classified as non-pyrogenic. When fever occurred in a test animal, the exudate was heated at 90°C. for 30 minutes and retested in the same dosage in at least one, usually two normal rabbits (depending upon the volume of exudate available). Ordinary bacterial pyrogens which might have contaminated the peritoneal fluids would not have been influenced by this treatment.

(c) *Lymph*—The dose of lymph employed in testing was also 5.0 ml., the number of animals used for each specimen depending upon the volume of lymph available. All negative tests were confirmed by injection of at least 10.0 ml. into another rabbit. Testing was repeated after heating the lymph at 90°C. for 30 minutes in every instance where fever resulted.

(d) *Extracts of Skin*—Each extract was divided into three equal parts, two being tested in normal rabbits and the third being heated at 90°C. for 30 minutes before testing to confirm the heat-lability of any pyrogenic substance found. The dosage of these extracts varied from 8.4 ml. to 17 ml./animal. In order to prevent sudden death in animals given these tissue extracts, a "desensitizing" dose (9) of 0.2 ml. was given 20 minutes before the full volume was injected and each animal received 50 mgm. of heparin intravenously immediately before the test.

Fever Index.—In several experiments, comparison of fever curves was facilitated by computing a *fever index*. Temperature records for 6 hours were drawn on standard graph paper and the areas beneath these curves were measured with a planimeter. The vernier reading of the planimeter was taken as the fever index, a numerical indication of height and duration of fever (10).

Avoidance of Pyrogen Contaminants.—Rigid precautions, similar to those described in previous reports (1, 3) were used to prevent contamination by ordinary bacterial pyrogens. These included testing of all solutions such as heparin, citrate, penicillin, etc. in rabbits, sterilization of glassware, needles, instruments, alundum, etc. in an oven for 2 hours at 170°C., and culture of test materials in thioglycollate broth. In the rare instances of contamination, materials were discarded.

Miscellaneous.—Purified endotoxin from *Shigella flexneri* was obtained from Dr. Leighton E. Cluff. Dr. Joseph Fruton kindly furnished crystalline preparations of various proteolytic enzymes, and cortisone was donated by Merck and Company as a suspension containing 25 mgm./ml. Dr. Robert R. Wagner supplied chick allantoic fluid infected with Type I pneumococci.

EXPERIMENTAL RESULTS

Limiting Experiments on the Effect of Injection of Pneumococci Upon Body Temperature of Normal Animals.—As is true of many Gram-positive cocci, the Type I pneumococcus has not been demonstrated to elaborate a pyrogenic toxin such as the heat-stable endotoxins of Gram-negative bacilli (11, 12). However, because all of the materials from infected animals in the present study contained pneumococci (usually killed by heat or with penicillin), it seemed desirable to obtain further information about the effect on body temperature of injection of these organisms alone into test animals. The following experiments were done in search of a pneumococcal pyrogen:

(a) Pneumococci were grown at 37°C. in tryptose phosphate broth, in trypticase soy broth, and in tryptose phosphate broth containing one per cent rabbit blood. After incubation for 12 hours, organisms were removed by filtration through Selas porcelain cylinders, washed with several volumes of buffered physiologic saline, and resuspended in saline. The number of viable bacteria in each suspension was determined by making agar pour plates of serial dilutions for colony counts. The intravenous injection of 2.7–8.4 $\times$ 10^9 viable pneumococci produced no immediate febrile response in normal rabbits although these animals later developed active infections with fever, usually beginning at the sixth to eighth hour after injection. When test animals were given 300,000 units of procaine penicillin intramuscularly 30 minutes before injection of bacterial suspensions, active infection was aborted and there was no elevation of body temperature.

(b) Because of the possibility that pneumococci multiplying in living tissues might elaborate some pyrogenic toxin that is not present in ordinary broth culture, embryonated chick eggs were inoculated with pneumococci and incubated at 37°C. for 16 hours. Infected allantoic fluids were collected and pooled. Normal allantoic fluid produced no fever when injected into test animals. Injection of 10 ml. of allantoic fluid containing 9.1 $\times$ 10^6 viable pneumococci/ml. produced no immediate febrile response in normal rabbits and late fever could again be avoided by prior administration of penicillin to test animals. Finally, pneumococci removed by filtration through Selas porcelain cylinders of peritoneal fluid collected from leukopenic rabbits with pneumococcal peritonitis produced no immediate fever in normal rabbits. The peritoneal fluid from rabbits made leukopenic with nitrogen mustard was used as a source of organisms because of its relative freedom from cellular debris and thick exudate. Leukopenic animals respond to this infection with high fevers (13).

In view of these results, which are detailed in Table I, it was felt that if fever-producing substances were found in animals with pneumococcal infection, their presence would probably not be attributable to simple elaboration of pyrogenic toxins by the infecting bacteria.

The Effect of Whole Blood or Serum From Rabbits with Pneumococcal Infection Upon the Body Temperature of Normal Rabbits.—The circulating blood of

TABLE I

Effect of Injection of Suspensions of Pneumococci Intravenously Into Normal Rabbits

Source of Organisms	No. Org. Injected	No. Test Animals	Fevers	
			Immediate	Late
Tryptose phosphate broth, 12 hr. culture	2.7×10^9	Normal 3	0	3
		*Penicillin 3	0	0
Trypticase soy broth, 12 hr. culture	3.8×10^9	Normal 3	0	3
		*Penicillin 3	0	0
Tryptose phosphate broth, blood added 12 hr. culture	8.4×10^9	Normal 5	0	5
		*Penicillin 5	0	0
Allantoic fluid	9.1×10^7	Normal 3	0	3
		*Penicillin 3	0	0
Peritoneal fluid, leukopenic rabbits	5.2×10^7	Normal 4	0	4
		*Penicillin 4	0	0

* 300,000 units procaine penicillin intramuscularly 30 minutes before injection of bacteria.

rabbits with fever due to pneumococcal peritonitis was tested for the presence of fever-producing substance by injection into normal animals.

Twenty-eight rabbits were inoculated intraperitoneally with pneumococci and their rectal temperatures were recorded at intervals of two hours. All were febrile by the eighth to tenth hour. Between 10 and 12 hours after initiation of infection, heparinized whole blood was collected from 8 animals, citrated whole blood from 8, and serum from 12. These materials were tested by intravenous injection into normal rabbits. In no instance was a febrile response elicited in a test animal. The rectal temperature before infection and temperature when sacrificed of infected donors is shown in Table II along with the quantities of blood and serum tested.

TABLE II

Blood and Serum of Rabbits with Type I Pneumococcal Peritonitis Tested for Presence of Fever-Producing Substance by Injection into Normal Rabbits

Donor Animals	Mean Rectal Temperature (°C)		Volume Collected*
	Before infection	When bled	
1–8 (Heparinized whole blood)	39.1	40.3	42 to 76 ml.
9–16 (Citrated whole blood)	38.8	40.2	54 to 80 ml.
17–28 (Serum)	39.2	40.8	34 to 60 ml.

* Penicillin added to each specimen in concentration of 10 units/ml. The dose injected into each test animal was one-half the volume collected. None of these bloods or serums produced fever in normal rabbits.

Failure to demonstrate the presence of circulating pyrogenic material in animals with pneumococcal peritonitis could be explained on the basis of inadequate quantities of blood used for testing. On the other hand, rapid removal or inactivation of pyrogens of tissue origin after intravenous injection into rabbits was found in a previous study in which factors of dosage were excluded (4).

Effect of Peritoneal Exudate.—An attempt was made to demonstrate fever-producing substance at the initial inflammatory site in rabbits with pneumococcal peritonitis.

Twelve rabbits were given intraperitoneal injections of a culture of pneumococci and their rectal temperatures were recorded at intervals of four hours. All were febrile by the twelfth hour after inoculation. Between 12 and 16 hours after infection, animals were sacrificed and peritoneal exudates were collected and tested by intravenous injection into normal rabbits. Ten of the exudates produced prompt, monophasic febrile responses in test animals in a dose of 5.0 ml. The other two exudates elicited similar responses when 20 ml. quantities were tested. The ability of peritoneal fluids to produce fever was regularly destroyed by heating at 90°C for 30 minutes. Figure 1 shows examples of the febrile response to pneumococcal exudate and the effect of heating. This experiment was repeated once with 12 rabbits and again with 18 rabbits with entirely similar results.

Effect of Thoracic Duct Lymph.—In view of failure to detect pyrogen in the circulating blood of infected rabbits, the possibility of absorption from the peritoneal cavity of fever-producing substance was explored further by testing for its presence in lymph draining the peritoneum.

A total of 36 rabbits with pneumococcal peritonitis was used. About 12–16 hours after infection, each animal was anesthetized with nembutal and thoracic duct lymph was collected for 30 to 60 minutes. At the end of this collection period, the animal was sacrificed, peritoneal

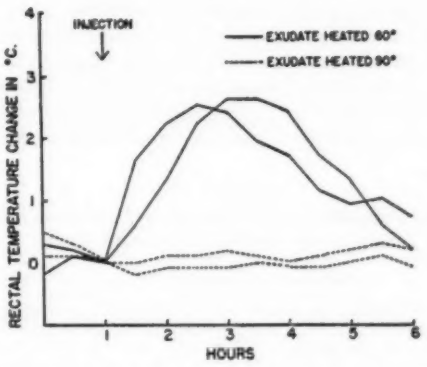


FIG. 1. Effect of intravenous injection of 5.0 ml. of pneumococcal peritoneal exudate upon body temperature of normal rabbits. Note the monophasic fevers with prompt rise and fall and the loss of fever-producing capacity when exudate is heated at 90°C. for 30 minutes.

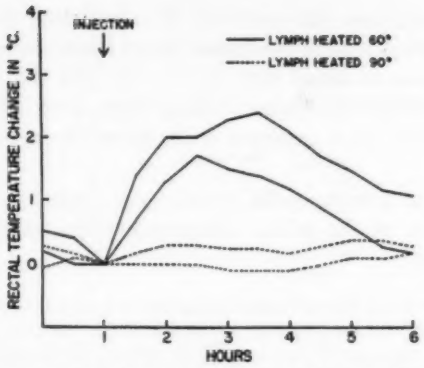


FIG. 2. Effect of intravenous injection of 5.0 ml. of thoracic duct lymph collected from animals with Type I pneumococcal peritonitis upon body temperature of normal rabbits.

exudate was obtained and both lymph and exudate were tested in normal rabbits by intravenous injection. In 26 instances, the volume of lymph collected exceeded 10.0 ml., making it possible to control results with specimens heated at 90°C. Of these 26 samples, 24 produced prompt febrile responses in a dose of 5.0 ml., examples of which are shown in Figure 2. The remaining two were inactive in this dosage. Heating at 90°C. for 30 minutes abolished the pyrogenic property (Fig. 2) of thoracic duct lymph.

Effect of Extracts of Dermal Pneumococcal Lesions.—In order to eliminate the possibility that the foregoing observations were due to some property peculiar to serous cavities, pneumococcal infection was initiated in another site and the area of inflammation was examined for fever-producing substances.

Dermal pneumococcal infection was produced in a group of 6 rabbits. At intervals of 24–28 hours after inoculation, when all were febrile and well-defined areas of hemorrhagic edema surrounded the inoculation site, animals were sacrificed, lesions and control areas of normal skin were excised and saline extracts were prepared. Five of the 6 extracts of inflamed skin produced febrile responses in test animals that were similar in all respects to the fevers described after injection of peritoneal exudate or thoracic duct lymph. None of the control extracts was pyrogenic. Heating at 90°C. destroyed the ability of extracts to produce fever. This experiment was repeated twice in groups of 6 rabbits with similar results. Table III

TABLE III

Extracts of Dermal Lesions Produced by Type I Pneumococcus Tested for the Presence of Fever-Producing Substance by Injection into Normal Rabbits

Donor Animals	Mean Rectal Temperature (°C)		No. extracts producing	
	Before Infection	When Sacrificed	*Fever	No Fever
D1-D6	38.9	41.1	5	1
D7-D12	39.5	41.2	6	0
D13-D18	39.3	40.7	4	2

* Each extract injected into two normal rabbits. Heating at 90°C. for 30 minutes destroyed pyrogenic activity of extracts.

details the findings with extracts of skin from these 18 animals with dermal pneumococcal infections.

Effect of Suppression of Febrile Response in Infected Animals Upon Appearance of Fever-Producing Substances in Exudate and Lymph.—The febrile response of rabbits with pneumococcal peritonitis could be suppressed by administration of amidopyrine or cortisone. Amidopyrine did not influence the course of the infection appreciably although cortisone, in the dosage employed, noticeably shortened survival time if the infection was allowed to run its full course. The impression was also gained that the peritoneal cavities of cortisone-treated animals contained less exudate than normals but this was not studied quantitatively. It can be said that smears of peritoneal washings from hormone-treated rabbits consistently showed numerous polymorphonuclear leukocytes and pneumococci and that the inflammatory process was never entirely suppressed. No attempt was made to explore dosage schedules for these antipyretic agents when it was found that the amounts used would prevent the development of fever in infected animals. It is entirely possible that smaller doses at longer intervals would have sufficed to obtain an equal antipyretic effect.

Six rabbits were inoculated intraperitoneally with Type I pneumococci and their rectal temperatures were recorded at intervals of 2 hours. Two animals had received no other medication; they became febrile at 6 to 8 hours. Two animals had been given 25 mgm. of cortisone daily by intramuscular injection for 3 days and were given 6.25 mgm. of the hormone immediately before injection of pneumococci and again 6 and 12 hours later. The remaining 2 were given 0.2 gm. of amidopyrine suspended in 0.5 ml. of physiologic saline subcutaneously beginning 2 hours before initiation of infection and injections were repeated at intervals of 4 hours for 5 doses. The animals given cortisone or amidopyrine remained afebrile. Their temperature curves for the first 16 hours after infection are shown in Figure 3. Twenty-two hours after injection of pneumococci, thoracic duct lymph and peritoneal

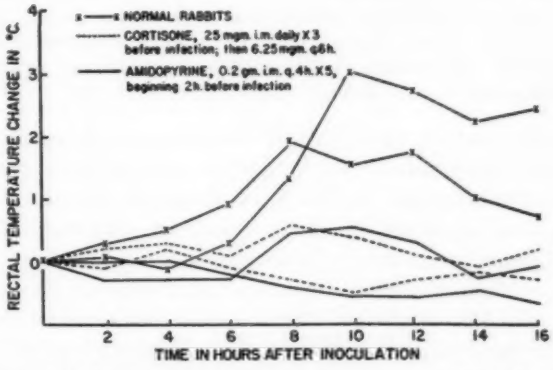


FIG. 3. Suppression of fever by cortisone or amidopyrine in rabbits after intraperitoneal inoculation of virulent Type I pneumococci.

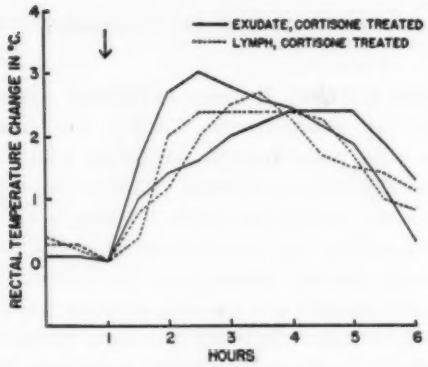


FIG. 4. Production of fever in normal rabbits by intravenous injection of 5.0 ml. of peritoneal exudate or thoracic duct lymph from cortisone-treated rabbits with Type I pneumococcal peritonitis.

TABLE IV

Peritoneal Exudate and Thoracic Duct Lymph of Rabbits Given Antipyretic Drugs Tested for the Presence of Fever-Producing Substance by Injection into Normal Animals

Infected Donor Animals	Mean Rectal Temperature (°C)		Pyrogen Test	
	Initial	When Sacrificed	Exudate	Lymph
A1-A6 (normal)	39.2	40.5	6 positive	6 positive
A7-A12 (amidopyrine*)	39.2	39.0	6 positive	5 positive‡
A13-A18 (cortisone†)	38.6	38.8	6 positive	6 positive

* 0.2 gram in 0.5 ml. physiologic saline subcut. q. 4 h. beginning 2 hr. before infection.

† 25 mgm. intramuscularly daily x 3 before infection; 6.25 mgm. immediately prior to infection and q. 6 h. x 3.

‡ Lymph was not collected from one animal given amidopyrine.

exudates were collected. Lymph and exudate from all 6 animals elicited characteristic febrile responses in test animals. The ability of these fluids to produce fever could be abolished by heating at 90°C. for 30 minutes. Figure 4 shows fever curves of normal rabbits given exudate and lymph from a cortisone-treated donor. This experiment was repeated twice with groups of 6 rabbits with the same findings, summarized in Table IV.

These results made it clear that if the fever-producing substance that appears at the inflammatory site in rabbits with pneumococcal peritonitis bears a causal relationship to the fever accompanying the infection, the antipyretic actions of amidopyrine and cortisone are not a result of suppression of its formation and must involve other pharmacologic mechanisms.

Properties of Fever-Producing Substances in Peritoneal Exudate, Thoracic Duct Lymph, and Skin Extracts of Rabbits with Pneumococcal Infections.—The ability of saline-induced peritoneal exudates and leukocyte extracts to produce fever in rabbits was shown in a previous study (2) to possess certain properties

including heat-lability, which clearly differ from those of ordinary bacterial pyrogens. Peritoneal exudate, thoracic duct lymph, and skin extracts of rabbits with pneumococcal infections were examined with respect to several of these properties:

(a) *Febrile response to single injection.*—With the exception that the duration of febrile responses after injection of pneumococcal exudate or lymph was more variable than those produced by leukocyte extracts or sterile exudates (2) the fevers were strikingly similar. The curves were monophasic and exhibited prompt rise and fall. In a total of more than 350 tests, a diphasic temperature curve of the type produced by bacterial pyrogens (2, 12) was never observed.

(b) *Febrile response to repeated injections.*—Daily injections of pooled exudate, pooled lymph, or pooled skin extracts were carried out for 15 days in groups of 4 normal rabbits, temperatures being recorded on days 1, 2, 4, 8, 11 and 15. As was the case with leukocyte extracts, there was no evidence of "tolerance" to the pyrogenic action of these materials (2) such as occurs with repeated injections of bacterial pyrogens (12). Table V illustrates the responses of these animals to daily injections.

(c) *Response of animals made tolerant to bacterial pyrogens.*—Because the acquired resistance to bacterial pyrogens conferred by daily injections is non-specific in the sense that it is operative against endotoxins from several species (10) the effect of such tolerance

TABLE V

Febrile Responses of Normal Rabbits to Daily Injections of Peritoneal Exudate, Thoracic Duct Lymph, or Extracts of Skin Lesions From Animals with Pneumococcal Infections

Animals	Material Injected	Fever Index					
		Day of Injection					
		1	2	4	8	11	15
E1	Peritoneal exudate	62	78	51	80	80	54
E2	Peritoneal exudate	37	58	62	56	41	49
E3	Peritoneal exudate	41	70	61	52	58	49
E4	Peritoneal exudate	66	98	72	*	54	82
	Mean	51.5	72.0	61.5	62.6	58.2	58.5
E5	Lymph	58	56	78	52	63	57
E6	Lymph	101	82	95	71	92	92
E7	Lymph	60	72	49	55	43	74
E8	Lymph	48	*	*	*	71	63
	Mean	66.7	70	74	59.3	67.2	71.5
E9	Skin extract	92	111	83	94	*	87
E10	Skin extract	43	66	37	52	35	60
E11	Skin extract	66	53	81	49	62	72
E12	Skin extract	84	101	90	84	84	96
	Mean	71.2	82.7	72.7	69.7	63.3	78.7

* Temperature record not obtained on this day.

upon febrile response to exudate and lymph was measured. Three normal rabbits were given pooled peritoneal exudate and three were given thoracic duct lymph on 2 successive days; their temperature responses were recorded and fever indices computed for a 6 hour period. On day 3, each animal began a series of 7 daily injections of 10 mcg. of Shigella endotoxin, fevers being measured on day 3 and again on day 9. As can be seen in Tables VI and VII, all had become relatively non-reactive to this bacterial pyrogen at the completion of this course of injections. On day 10, injection of exudate or lymph was repeated; fever indices were similar to those on days 1 and 2, indicating no cross-tolerance. Finally, administration of 6.0 ml. of Thorotrast, a procedure known to abolish pyrogen tolerance and to render animals far more susceptible to the action of bacterial endotoxin (14), was

TABLE VI

Responses of Rabbits Made Tolerant to Bacterial Pyrogens to Injection of Peritoneal Exudate

Animal	Fever Index					
	Material injected					
	Ex.	Ex.	End.	End.	Ex.	Ex.
	Day					
	1	2	3	9*	10	11†
T-1	28	47	137	42	74	71
T-2	55	36	99	67	42	61
T-3	42	61	114	28	50	46
Mean	41.6	48	116.6	45.6	55.3	59.3

Ex. = 5.0 ml. peritoneal exudate.
End. = 10 mcg. Shigella endotoxin.
* Daily injection Shigella endotoxin, days 3–9.
† All animals given 6.0 ml. Thorotrast intravenously 2 hr. before injection of exudate.

TABLE VII

Responses of Rabbits Made Tolerant to Bacterial Pyrogens to Injection of Thoracic Duct Lymph

Animal	Fever Index					
	Material injected					
	Ly.	Ly.	End.	End.	Ly.	Ly.
	Day					
	1	2	3	9*	10	11†
T-4	22	36	141	47	31	28
T-5	44	69	107	31	71	49
T-6	55	40	128	70	61	44
Mean	40.3	48.3	125.3	49.3	54.3	40.3

Ly. = 5.0 ml. thoracic duct lymph.
End. = 10 mcg. Shigella endotoxin.
* Daily injection Shigella endotoxin, days 3–9.
† All animals given 6.0 ml. Thorotrast intravenously 2 hr. before injection of lymph.

without appreciable influence upon the febrile responses to exudate or lymph of these rabbits on day 11.

(d) *Effect of heat and pH.*—The ability of exudate and lymph samples to produce fever was retained over a pH range of 2.5 to 10.0. This range resembles that found for leukocyte extracts and sterile exudates (2). Boiling for 5 minutes or heating at 90°C. regularly destroyed the ability of peritoneal exudates, thoracic duct lymph, and dermal extracts to produce fever. Exudates and lymph retained ability to produce fever after storage at 4°C. for 15 months. The influence of pH upon heat lability of these materials was also tested. At pH 4.5, ability to cause fever was abolished by heating at 70°C. for 30 minutes. At pH 7.2, ability to cause fever remained intact at 70°C. but was destroyed regularly by heating at 85°–90° for 30 minutes. This effect was entirely similar to that previously observed with leukocyte extracts and sterile exudates (2).

(e) *Effect of dialysis.*—After dialysis through cellophane for 7 days against distilled water or physiologic saline with daily changing of the bath, the fever-producing material of exudates and of lymph remained within the dialysis bag.

(f) *Effect of proteolytic enzymes.*—As was the case with leukocyte extracts (2), digestion with trypsin, chymotrypsin, or ribonuclease was without effect upon the pyrogenic property of pneumococcal exudates or lymph. Treatment with these enzymes did not cause the fever-producing substance in these materials to become dialyzable.

(g) *Effect of amidopyrine, cortisone, heparin, and penicillin upon febrile response.*—Prior treatment of normal rabbits with cortisone (25 mgm. intramuscularly daily for 3 days, then

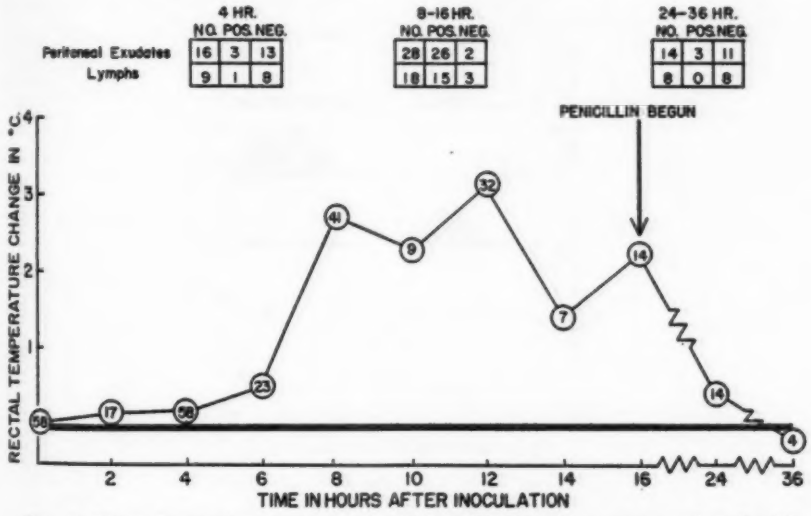


FIG. 5. Mean temperature response of rabbits with Type I pneumococcal peritonitis. The figures in circles indicate the number of temperature readings recorded and averaged at each interval. Groups of animals were sacrificed at 4 hours, 8–16 hours, and 24–36 hours and peritoneal exudates and thoracic duct lymph specimens collected and tested for ability to produce fever. The number of specimens obtained and the results of testing are listed in the boxes at the top. The 14 animals remaining at 16 hours were treated with penicillin and sacrificed when they had become afebrile.

6.5 mgm. every 6 hours) or with amidopyrine (0.2 gm. subcutaneously every 4 hours) suppressed the febrile response to exudate or lymph from animals with pneumococcal infection. Heparin in doses up to 100 mgm. intravenously or penicillin (600,000 units procaine penicillin G) were without effect upon the febrile response.

All of these results correspond closely to observations made under similar conditions with leukocyte extracts or with the fluid portion of saline-induced peritoneal exudates (2). None of the properties tested, of course, is of such specificity as to justify any conclusions about identity of the pyrogenic substances, although it is clear that they differ in several respects from ordinary bacterial pyrogens.

Correlation of Fever-Producing Substances in Peritoneal Exudates and Lymph with Febrile Course of Rabbits with Pneumococcal Peritonitis.—The temperature response of rabbits with pneumococcal peritonitis was rather consistent. Animals rarely became febrile before the eighth hour after inoculation of bacteria

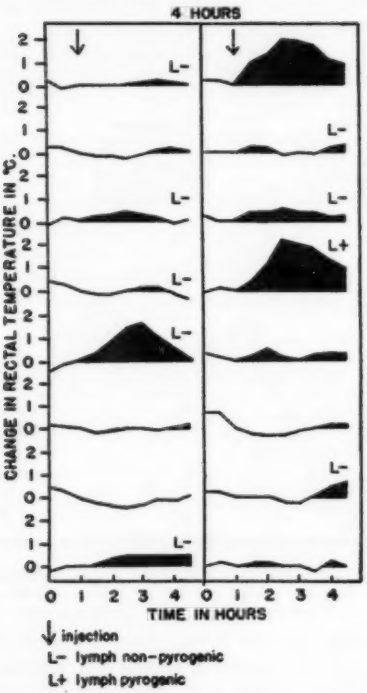


FIG. 6. Temperature curves of test animals injected with peritoneal exudate from 16 rabbits sacrificed 4 hours after intraperitoneal injection of virulent Type I pneumococci. Each curve is the mean of the responses of 2 normal rabbits. Lymph specimens were also collected and tested from 9 of the 16 animals.

into the peritoneal cavity. Thereafter, they ran high fevers until death, which usually occurred 24–36 hours after infection, although an occasional rabbit succumbed as early as the 18th hour. This infection could usually be controlled by administration of penicillin as late as the 16th hour. When penicillin was given, there was prompt defervescence, temperature returned to normal by the twenty-fourth to thirty-sixth hour and animals survived. The following experiment was carried out in an attempt to correlate the clinical course of fever in pneumococcal peritonitis with the presence of fever-producing substances at the inflammatory site.

Fifty-eight rabbits with pneumococcal peritonitis were used. They were infected in groups of 6 or 10, and rectal temperatures were recorded every 2–4 hours. At various times after infection, animals were sacrificed and peritoneal exudate was collected. In most

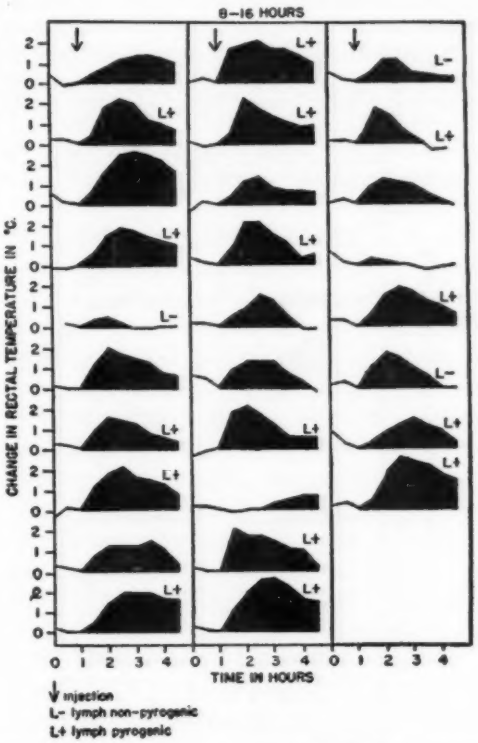


FIG. 7. Mean temperature responses of pairs of animals injected intravenously with peritoneal exudates collected from 28 rabbits 8–16 hours after intraperitoneal injection of virulent Type I pneumococci. Lymph specimens were collected and tested from 18 of the 28 animals.

instances, it was also possible to obtain thoracic duct lymph in quantities large enough to allow testing. Animals surviving to the sixteenth hour were given a single initial injection of 25,000 units of aqueous penicillin G intraperitoneally and 50,000 units intramuscularly every 4 hours until sacrificed. Figure 5 illustrates the mean temperature curve of the 58 rabbits. Sixteen animals were sacrificed after 4 hours, 28 after 8–16 hours, and 14 after 24–36 hours when they had become afebrile as a result of treatment with penicillin. Peritoneal exudate was collected from every animal; the number of specimens of thoracic duct lymph collected at each interval was: 4 hours, 9 samples; 8–16 hours, 18 samples; 24–36 hours, 8 samples. Each specimen of exudate or lymph was tested by injection of 5.0 ml. into 2 normal rabbits. Each positive result was checked by repeating the test after heating the sample at 90°C. for 30 minutes. Each negative result was confirmed by injecting at least 10 ml., usually 20 ml. into another animal. The results of this experiment are depicted graphically in Figures 6–8. With occasional exceptions, exudate and lymph at 4 hours were non-pyrogenic, after 8–16 hours they contained heat-labile pyrogen, and after control of the infection, specimens were again non-pyrogenic. It should be mentioned that the peritoneal cavities of animals treated with penicillin still contained large amounts of exudate which showed few or no organisms in stained smears but many polymorphonuclear leukocytes. Cultures were always negative for pneumococci (penicillinase was not added to culture media). The leukocytes appeared to be degenerating, many free granules were found in smears, and in several animals, mononuclear cells had become prominent in the exudate.

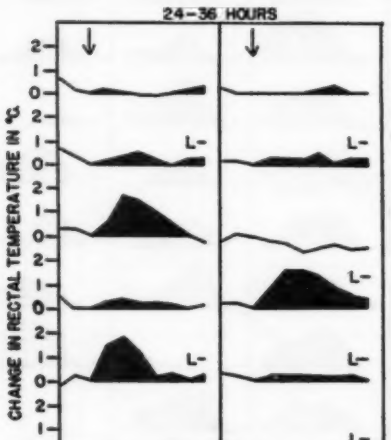


FIG. 8. Responses of test rabbits to intravenous injection of peritoneal exudate from 14 animals treated with penicillin 16 hours after injection of virulent Type I pneumococci intraperitoneally and sacrificed when afebrile. Thoracic duct lymph from 8 of the 14 animals was also tested.

It is clear from these findings that with the onset of fever in the course of pneumococcal peritonitis in rabbits, there appears in the exudate a heat-labile pyrogen that is also demonstrable in lymph draining the inflamed area. With the exhibition of penicillin and subsidence of fever, the pyrogenic substance is no longer demonstrable in the peritoneum or thoracic duct lymph.

DISCUSSION

Fever is a common accompaniment of many diseases including infections, neoplasms, vascular occlusions, sensitivity states, and metabolic abnormalities. The mechanisms responsible for disturbance in regulation of body temperature in these disorders are poorly understood. Because destruction of tissue is common to all these afflictions, the hypothesis that some product of injured cells acts upon neural centers controlling heat balance is widely accepted but has by no means been established. This explanation is a reasonable one and has the further appeal of being susceptible to experimental study. As has been pointed out in detail in a previous report (1), the evaluation of earlier studies on fever-producing substances of presumed tissue origin is difficult because many of the reported findings could have resulted from contamination of test-materials with ordinary bacterial pyrogens. In certain investigations, this has undoubtedly been the case.

That endogenous substances capable of causing fever occur in the tissues of the rabbit seems established. Extracts of polymorphonuclear leukocytes, the fluid portion of sterile peritoneal exudates in normal or leukopenic rabbits, and extracts of localized inflammations such as the Arthus reaction have the capacity of inducing fever when injected into test animals (1, 2). Atkins and Wood (15) have recently described a pyrogenic material in the blood of rabbits given typhoid vaccine intravenously that differs in several respects from the originally injected bacterial pyrogen.

The *source* of endogenous pyrogenic substances in the rabbit has not been defined completely. The finding of pyrogenic activity in sterile exudates and extracts of leukocytes in a prior study (1) seemed to offer a partial answer. However, the fact that peritoneal fluid collected from neutropenic rabbits is also capable of producing fever makes it difficult to conclude that the leukocyte is the only source of endogenous fever-producing substances (2). Surveys of the tissues of normal (1) and leukopenic (3) rabbits have failed to reveal another source. Such tests are of limited significance, however. Not only is it conceivable that the several methods employed for extracting tissues were inadequate or the quantities used too small, but there is also the possibility that under conditions of injury or irritation, cells may elaborate pyrogenic substances not normally present in detectable amounts. Atkins and Wood (15) have suggested that the fever-producing material found by them in the circulating blood of rabbits given typhoid vaccine may originate, at least in part,

from injured leukocytes. The regular occurrence of transient leukopenia before the febrile response in rabbits given bacterial endotoxins would fit with this suggestion (16). More important, these investigators have recently shown that animals with established tolerance to bacterial pyrogens are capable of reacting to injections of the serum pyrogen with fevers as high as those of normal rabbits (17). The febrile response produced by this material is monophasic, brief, and in all respects similar to that resulting from injection of leukocyte extracts (2) or the pneumococcal exudates used in the present study. Because the endogenous pyrogen that appears in rabbits after injection of typhoid vaccine has thus far been identified only in serum, it has been technically impossible to test its lability to heat (17). Although it must be recognized that there remains a possibility that Wood and Atkins are dealing with the typhoid endotoxin that has been modified by contact with the hosts' tissues in some way that alters remarkably its original properties, all of the evidence now available points to an endogenous source for the circulating pyrogen that appears in rabbits given typhoid vaccine.

Transient granulocytopenia has been observed to precede febrile responses induced in rabbits by stimuli other than bacterial endotoxins including hypersensitivity reactions of the immediate (18, 19) or delayed (20) type and the injection of such substances as kaolin and glycogen (21). While this may be regarded as corroborative evidence in support of the idea that release of a pyrogen from leukocytes is a pathogenetic factor common to fevers of several types, it does not constitute additional proof. The febrile reaction of rabbits to injection of influenzal viruses is unaccompanied by granulocytopenia (22) and the rabbits made neutropenic with nitrogen mustard respond with fevers as high as those of normal animals to pneumococcal infections (13), hypersensitivity of the anaphylactic type whether actively or passively induced (19), injection of bacterial endotoxins (23), injection of influenza virus (24), and injection of kaolin or glycogen (21). It is also, of course, well-known that patients with agranulocytosis are capable of responding to infections with high fevers.

Finally, granted that endogenous substances capable of causing fever when injected into *other animals* are demonstrable in the several situations mentioned above, the question of their role in the production of fever in the *host or donor animal* remains. It is this aspect of the problem that prompted the present investigation of pneumococcal infection in the rabbit. It can now be said that the onset of fever in rabbits infected with Type I pneumococci is accompanied by the appearance at the initial inflammatory site of a heat-labile fever-producing substance. This pyrogen is also demonstrable in thoracic duct lymph, indicating its access to the venous circulation. When infection is terminated by administration of penicillin and fever subsides, the pyrogen is

no longer found in exudate or lymph. Although efforts to demonstrate fever-producing substance in the circulating blood of rabbits with pneumococcal infections have been unrewarding to date, it seems inescapable that the pyrogen in thoracic duct lymph enters the circulation. The data do not permit the conclusion that the fever accompanying pneumococcal infection in the rabbit is caused by the substance. The parallelism, however, is striking and highly suggestive of a causal relationship. Furthermore, the similarity of the properties of the pyrogen in pneumococcal exudates and fever-producing substances found at sterile inflammatory sites in previous studies (1, 2) suggests that the fever-producing substance in pneumococcal peritonitis originates from the tissues of the host. The properties that have been studied, however, are so non-specific as to preclude further comment. Finally, it is pertinent to point out that another parallelism existed in the experiments reported here; the *onset* of fever correlated nicely with the *multiplication of infecting bacteria* in the hosts' tissues and the *subsidence* of fever with the *destruction of the microörganisms* by penicillin. Although it was not possible by the methods employed to demonstrate a pyrogen in the pneumococcus, it cannot be said that this possibility has been entirely excluded. It is hoped that studies now in progress will lead to specific characterization of these various pyrogenic substances and permit more definite conclusions about their sources and similarities.

SUMMARY

At various stages of Type I pneumococcal infection in rabbits, blood, inflammatory exudate, and thoracic duct lymph were collected and tested for the presence of fever-producing substances by intravenous injection into normal rabbits. It was found that the onset of fever in pneumococcal peritonitis is accompanied by the appearance of a heat-labile pyrogen at the site of the initial lesion and in lymph draining the area. By the methods employed, no pyrogenic activity was found in blood from infected animals. With termination of infection by penicillin and subsidence of fever, the pyrogen disappeared from exudate and lymph. No pyrogen was demonstrable in the bacteria themselves and the properties of the fever-producing substances in pneumococcal exudates corresponded closely to those of previously described tissue pyrogens. Amidopyrine and cortisone suppressed fever in infected animals but did not prevent the appearance of pyrogen in lymph and exudate. The relationship of these findings to other studies pointing to products of tissue injury as a cause of the fever accompanying various disease states is discussed. It is concluded that while the fever of pneumococcal infection in rabbits could be explained by the pyrogen which appears in exudate and lymph, further investigation aimed at more specific characterization of "endogenous" pyrogens is needed to support a causal relationship.

ACKNOWLEDGMENT

The author is grateful to Mrs. Catherine Wichhart and Miss Elizabeth Roberts whose skilled technical assistance made these studies possible.

BIBLIOGRAPHY

1. Bennett, I. L., Jr. and Beeson, P. B.: Studies on the pathogenesis of fever I. The effect of injection of extracts and suspension of uninfected rabbit tissues upon the body temperature of normal rabbits. J. Exper. Med., 1953, **98:** 477.
2. Bennett, I. L., Jr. and Beeson, P. B.: Studies on the pathogenesis of fever II. Characterization of fever-producing substances from polymorphonuclear leukocytes and from the fluid of sterile exudates. J. Exper. Med., 1953, **98:** 493.
3. Bennett, I. L., Jr.: Studies on the pathogenesis of fever III. Failure to demonstrate fever-producing substances in the tissues of leukopenic rabbits. Bull. Johns Hopkins Hosp., 1956, **98:** 1.
4. Bennett, I. L., Jr.: Studies on the pathogenesis of fever IV. Further observations on the production of fever in rabbits by the fluid of sterile exudates. Bull. Johns Hopkins Hosp., 1956, **98:** 7.
5. Goodner, K.: Experimental intradermal pneumococcus infection in rabbits. J. Exper. Med., 1928, **48:** 1.
6. Schulz, R. Z., Warren, M. F. and Drinker, C. K.: The passage of rabbit virulent type III pneumococci from the respiratory tract of rabbits into the lymphatics and blood. J. Exper. Med., 1938, **68:** 251.
7. Smith, R. O. and Wood, W. B., Jr.: Cellular mechanisms of antibacterial defense in lymph nodes I. Pathogenesis of acute bacterial lymphadenitis. J. Exper. Med., 1949, **90:** 555.
8. Smith, R. O. and Wood, W. B., Jr.: Cellular mechanisms of antibacterial defense in lymph nodes II. The origin and filtration effect of granulocytes in the nodal sinuses during acute bacterial lymphadenitis. J. Exper. Med., 1949, **90:** 567.
9. Thomas, L.: Studies on the intravascular thromboplastic effect of tissue suspensions in mice I. The reaction of mice to intravenous injections of a sedimentable tissue component. Bull. Johns Hopkins Hosp., 1947, **81:** 1.
10. Beeson, P. B.: Tolerance to bacterial pyrogens I. Factors influencing its development. J. Exper. Med., 1947, **86:** 29.
11. Bennett, I. L., Jr.: Observations on the fever caused by bacterial pyrogens I. A study of the relationship between the fever caused by bacterial pyrogens and the fever accompanying acute infections. J. Exper. Med., 1948, **88:** 267.
12. Bennett, I. L., Jr. and Beeson, P. B.: The properties and biologic effects of bacterial pyrogens. Medicine, 1950, **29:** 265.
13. Bennett, I. L., Jr.: Studies on the pathogenesis of fever. Fever accompanying pneumococcal infections in leukopenic rabbits, in preparation.
14. Beeson, P. B.: Tolerance to bacterial pyrogens II. Role of the reticuloendothelial system. J. Exper. Med., 1947, **86:** 39.
15. Atkins, E. and Wood, W. B. Jr.,: Studies on the pathogenesis of fever I. The presence of transferable pyrogen in the blood stream following the injection of typhoid vaccine. J. Exper. Med., 1955, **101:** 519.
16. Atkins, E., Allison, F., Jr., Smith, M. R. and Wood, W. B., Jr.: Studies on the antipyretic action of cortisone in pyrogen induced fever. J. Exper. Med., 1955, **101:** 353.

17. Atkins, E. and Wood, W. B., Jr.: Studies on the pathogenesis of fever II. Identification of an endogenous pyrogen in the blood stream following the injection of typhoid vaccine. J. Exper. Med., 1955, **102:** 499.
18. Farr, R. S., Campbell, D. H., Clark, S. L., Jr. and Proffitt, J. E.: The febrile response of sensitized rabbits to the intravenous injection of antigen. Anat. Rec., 1954, **118:** 385 (abstract).
19. Bennett, I. L., Jr.: Studies on the pathogenesis of fever. The role of anaphylactic hypersensitivity, in preparation.
20. Stetson, C. A., Jr.: Studies on the mechanism of the Shwartzman phenomenon. Similarities between reactions to endotoxins and certain reactions of bacterial allergy. J. Exper. Med., 1955, **101:** 421.
21. Bennett, I. L., Jr.: unpublished observations.
22. Wagner, R. R., Bennett, I. L., Jr. and LeQuire, V. S.: The production of fever by influenzal viruses I. Factors influencing the febrile response to single injections of virus. J. Exper. Med., 1949, **90:** 321.
23. Bennett, I. L., Jr. and Cluff, L. E.: Influence of nitrogen mustard upon reactions to bacterial endotoxins: Shwartzman phenomenon and fever. Proc. Soc. Exper. Biol. & Med., 1952, **81:** 304.
24. Bennett, I. L., Jr. and Wagner, R. R.: Production of fever by influenzal viruses V. Effect of nitrogen mustard (HN_2). Bull. Johns Hopkins Hosp., 1955, **97:** 43.

ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

December 12, 1955

RADIOTHERAPY OF SUPERFICIAL EPITHELIOMATOSIS

MARK HOLLANDER

Superficial epitheliomatosis is a fairly common variant of basal cell epithelioma whose growth potential appears to be horizontal, parallel to the skin surface, and usually to remain so for 20–30 years. Consequently, a tumor can spread out to form a plaque varying up to 20–30 cm. in diameter without going deep or ulcerating. At this stage, the lesion feels quite superficial, and is often misdiagnosed as tinea circinata, dermatitis, or psoriasis. The thread-like pearly nodular border, however, is pathognomonic. Breakthrough with deep invasion can occur at any time, usually as basal cell epithelioma but infrequently as prickle cell carcinoma. When this does occur, palpable nodules appear and may ulcerate. The plaques are usually multiple.

Histologically, the tumor consists of masses of basal-type cells with hyperchromatic nuclei extending down into the papillary and subpapillary layers of the cutis from multiple foci of origin at the tips of interpapillary pegs. The reticular layer is not encroached upon, and fibrosis occurs only as a result of excoriation or infection. The tumor usually remains thus superficial and non-ulcerative for many years.

Superficial epitheliomatosis is represented by the dermatologic textbooks (1) and literature as being radioresistant, despite the known radiosensitivity of the cellular types of basal cell epithelioma. This is apparently due to the high incidence of severe radiation sequelae following treatment of the tumors with conventional x-rays.

The surface dose and percentage depth dose of conventional x-rays increase rapidly as the field is opened, because of backscatter (2). The variations with field size for unfiltered 100 KV x-rays, as commonly used by determatologists, and for 120 KV x-rays with 3 mm. Al added filter, the softest beam generally used by radiologists in the treatment of skin lesions, are shown in chart 1. These data show that the air dose which would deliver about 5000 r to a 1 cm. epithelioma would amount to about 6700 r on a plaque 10 cm. in diameter, so that the apparent radioresistance of superficial epitheliomatosis could be merely an expression of overdosage.

Grenz rays are x-rays (3) generated in the range of 8–15 KVP. Since backscatter has not been detected in this range, air dose and skin dose are identical for fields of any size. This suggested that it might be possible to treat superficial epitheliomatosis successfully with these very soft rays. The usual depth of the tumor masses is not more than about 0.5 mm., so medium Grenz rays (curve #4, fig. 1) appeared to offer adequate depth dosage. The depth dose of this quality of radiation is only about 3.5 per cent at 3 mm., so that high surface dosage should not cause material deep damage to the skin.

Experimental irradiation of plaques of superficial epitheliomatosis was started in September 1951. The first three lesions were treated fractionally, at intervals of 3–4 days. All others had single massive dose therapy. All of 14 tumors receiving 3000 r or more disappeared. Two lesions receiving 2000 r recurred within a month or two. Plaques given fractional therapy responded as well as did those receiving the same total dose at one sitting. Eleven successfully-treated tumors have been followed for four years or more, and three have been followed for eight months, without recurrence. Superficial atrophy and telangiectasia have occurred in several instances, but no evidence of deep or severe radiation injury has developed, even in a

CHART 1

Roentgens at Depth per 100 r in Air

Area—Sq. cm. (Diam.—cm.)	5 (2.5)	75 (10)
Depth—mm		
H.V.L. 1 mm. Al (about 100 KV, inherent filter only)		
0	106	114
5	68	85
H.V.L. 4 mm. Al (about 120 KV, 3 mm. Al added filter)		
0	114	132
5	96	121

Results of Treatment

Number of Lesions	Dose	Result
	r	
1	8 × 500	Healed, four years
1	5 × 1000	Healed, four years
1	5 × 1500	Healed, four years
1	7500	Healed, four years
3	5000	Healed, 3–4 years
3	5000	Healed, 6 months
3	4000	Healed, 3½ years
1	3000	Healed, 3½ years
2	2000	Recurrence within a month or two

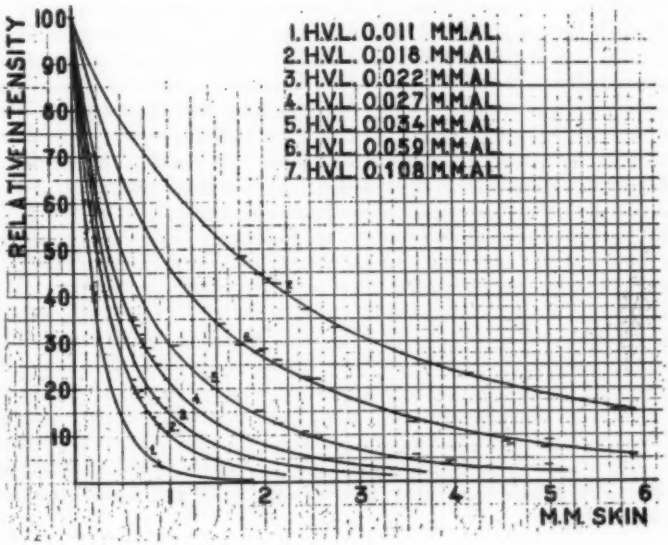


FIG. 1

lesion exposed to 7500 r in a single dose. The best cosmetic results were seen in areas exposed to 4000 or 5000 r in fractional dosage.

Biopsies taken from several tumor sites three months post-treatment showed mild radiation changes in the epidermis and questionable changes in the upper cutis. No tumor cells were found.

In summary, superficial epitheliomatosis is a radiosensitive neoplasm which responds well to medium Grenz rays. Optimal dosage seems to be 4–5 × 1000 r, at intervals of 3–4 days. In a follow-up period of four years, no lesion receiving 3000 r or more has recurred, while tumors receiving 2000 r recurred within a month or two. Doses as high as 7500 r in one exposure have caused superficial atrophy and telangiectasia, but no deeper or more extensive sequelae. It is suggested that intraepithelial carcinomata should respond to this therapy.

REFERENCES

1. Ormsby, O. S. and Montgomery, H.: *Diseases of the Skin*, 7th edition. Lea & Febiger, Philadelphia, Pa., 1948.
2. Glasser, O., Quimby, E. H., Taylor, L. S., and Weatherwax, J. L.: *Physical Foundations of Radiology*, Paul B. Hoeber, Inc., New York, N. Y., 1945.
3. Hollander, M. B.: Grenz rays, J. Invest. Dermat. 1953, **21:** 15–25.

FAMILIAL PRIMARY AMYLOIDOSIS

Lawrence E. Shulman and Frederick C. Bartter

The case histories of three members of the same family—mother (38 years of age), sister (34 years) and brother (24 years)—with histologically "proven" amyloidosis were presented. The manifestations of illness were atypical and differed from those seen in secondary amyloidosis complicating chronic disease states and also from those usually found in so-called primary amyloidosis wherein cardiovascular involvement predominates in older individuals.

The clinical pictures in the mother and daughter were virtually identical and resembled in a striking manner that described in 1952 by the Portuguese neurologist, C. Andrade, (Brain, **75:** 408). He emphasized the special involvement of the peripheral nerves and observed the disease to be endemic among seventy-four individuals residing in a circumscribed fishing area along the coast of Portugal. Most of his cases occurred in family groups. In our patients, as in his, the disease began insidiously and progressed relentlessly to death over a three to five-year period. The patients became weak, nervous and began to lose weight. Prolonged episodes of nausea and vomiting were followed by periods of alternating diarrhea and constipation and finally fecal incontinence. They then developed progressive numbness and weakness first of the lower extremities and subsequently, of the upper extremities as well. Both complained of staggering episodes and blacking-out spells. Libido and menstruation ceased. Distinctive features on physical examination were severe postural hypotension, widespread ecchymoses, pupils reacting not at all or sluggishly both to light and in accommodation, slight to moderate enlargement of the liver and spleen and evidence of a severe peripheral neuritis particularly in the lower extremities and manifested by severe muscular wasting, sluggish or absent deep tendon reflexes and loss of sensation. Laboratory abnormalities included anemia, elevated cephalin-cholesterol flocculation and an elevated protein concentration in the cerebro-spinal fluid. The Congo Red test was equivocally positive. Amyloidosis, confirmed by metachromatic staining, was found in several organs, but of particular interest was the extensive deposition in the peripheral nerves. The relationship between the amyloid deposits in the nerve fibers and the nutrient vessels could not be ascertained.

The illness in the brother has to date been different from that seen in his mother and sister. In 1954 he was admitted because of the acute onset of blindness, virtually complete on the right and incomplete on the left. Examination revealed the vitreous to be filled with linear opacities, appearing much like glass wool. The pupils were normal. Extensive laboratory investigation was non-revealing. During observation, however, the patient developed rapidly a walnut-sized mass in the right lobe of the thyroid which was removed and found to be an amyloid goitre.

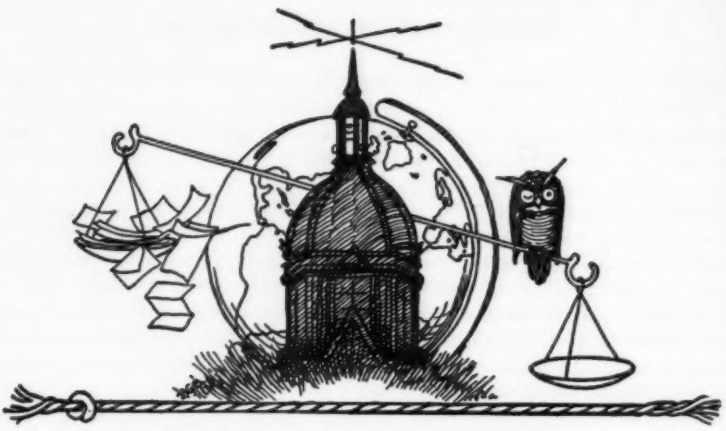
The disease in our family is inherited as a Mendelian autosomal dominant. It is of interest that the brother and sister had different fathers. Investigation of the family reveals that there are three younger asymptomatic siblings. However, according to Andrade the disease does not usually begin to express itself until the third or fourth decades. As far as could be determined, a similar illness had not occurred among our patients' antecedents who were of Dutch and German origin several generations ago.

A review of the literature has strongly suggested that many patients reported to have had the progressive hypertrophic polyneuritis of Dejerine and Sottas may well have had this familial form of amyloidosis.

STUDIES ON MERCURIAL DIURETICS

GILBERT H. MUDGE

Many studies have indicated that the diuresis produced by organic mercurials may be augmented by high-chloride acidosis and be prevented by hypochloremic alkalosis. These relationships have previously been interpreted as indicating that the mercurial refractoriness of alkalosis was attributable to the diminished amount of chloride filtered at the glomerulus. The present experiments were initiated on the hypothesis that the interaction of a mercurial with a cellular component might be sensitive to changes in pH. They were carried out in collaboration with Mr. Byron Hardin. The action of mercurials was determined during two types of alkalosis, first sodium bicarbonate infusions (extra- and intra-cellular alkalosis), and secondly, chronic potassium depletion (extracellular alkalosis, intracellular acidosis). No chloruresis was obtained when organic mercurials were administered to dogs made alkalotic by bicarbonate infusions. The alkalosis of potassium depletion was characterized by comparable changes in the composition of the extracellular fluid, but the administration of mercurials was followed by a normal, or possibly an augmented, diuretic response. Since the glomerular filtration rate was the same in both types of alkalosis, the results indicate that factors other than the filtered chloride load must determine the diuretic effectiveness of organic mercurials. Further studies are in progress to study the relationship of pH to the effect of organic mercurials on cellular metabolism in isolated systems.



BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Differential Diagnosis. The Interpretation of Clinical Evidence. By A. McGehee Harvey and James Bordley, III. 665 pp., $11.00. W. B. Saunders Co., Philadelphia, Pa.

For years the Wednesday Clinical-Pathological Conferences at The Johns Hopkins Medical School have afforded participants and audience alike exhilarating experience in that most challenging of medical disciplines, differential diagnosis. This experience has been unique not only because of the intriguing character and wide variety of the clinical problems presented, as well as the inspiring brilliance of many of the discussions, but also because seldom has there been so well formulated the intellectual approach a physician should have to diagnostic problems. Many a student, enthralled by the incisive reasoning of Hamman and the soundly logical approach of Harvey making their conclusions seem so inevitably correct, has wished that all this wisdom of experience in differential diagnosis would be given substance in print. This has now been exceedingly well accomplished in this volume by Harvey and Bordley. As pointed out in the preface, Hamman should also be regarded almost as a co-author, so considerable has his posthumous contribution been to the spirit and content of this book. He could be well satisfied, we think, with what has been accomplished with his legacy.

This book is not a compendium of the signs and symptoms encountered in a systematic review of various disease states with their differentiating features. It is not a textbook. It is a presentation of a method of approach to the diagnosis of disease. After describing this method and the principles upon which it is based, the authors then apply it to a variety of commonly encountered clinical situations. In each instance a number of illustrative cases are presented, consisting of previous clinical-pathological conferences, the clinical discussions being unaltered from the original and the autopsy findings being briefly outlined. In the last chapter a group of "unknowns" affords the reader an opportunity to test his acumen with this diagnostic method. There are a number of highly useful tables in which are categorized in an unique manner certain common manifestations of disease. The book closes with a helpful general index, and also a special index of symptoms, signs and laboratory findings. The whole book is well and tastefully printed, with a minimal smattering of typographical errors.

As already indicated, this book will please anyone, physician or student, who has felt himself enriched and stimulated by the Wednesday Clinical-Pathological Conferences, for it contains the same ingredients of soundness of approach, beauty of logic, and clarity of expression that has made them superb. It is a book which must be studied and not simply perused, as it contains an appreciable quantity of valuable, fundamental, clinical information which is not elsewhere to be found, and the diagnostic method which it proposes, like all disciplines, has to be learned. There is a great deal of wisdom in this book, and much that is practically helpful. Read and re-read, and its diagnostic method made part of daily practice, this book will have an important influence upon the clinical habits and thinking of physicians for years to come.

PHILIP A. TUMULTY

Mental Hygiene in Public Health, 2nd edition. By PAUL V. LEMKAU. 486 pp., $8.00. McGraw-Hill Book Co., New York, N. Y.

This is the second edition of a work written primarily as a textbook for students of public health. As such it is devoted to the conjunction of psychiatry and the problems of public health. The smaller type and increased number of pages testify to a considerable enlargement of this second edition; it is well printed and the new format is an improvement.

The first part of the book, titled "The Place of Mental Hygiene in Public Health," has been largely rewritten and expanded. The new material describes the methods and procedures of a program of mental hygiene and considers various modes of attack on the problems involved in setting up a mental hygiene program in a community. For public health officers and others engaged in such activities the new information presented in this section will more than justify the publication of this new edition at this time.

The second part of the book, titled "The Development of the Individual," appears little changed from the first edition. This entire section is unusually sound and well written and could serve with distinction as a reference source for the student of general medicine as well as the student of public health. Notably praiseworthy is the author's avoidance of objectionable psychiatric and psychoanalytic jargon in discussing personality development, which can only enhance the usefulness and intelligibility of these chapters. Accuracy has not been compromised.

Although the book is not intended to be a textbook of psychopathology, there is a brief section in an appendix which reviews morbid signs and symptoms as well as current psychiatric nosology. The concluding chapter, titled "Visual Aids," has been brought up to date and is a useful source of information for those engaged in planning mental health programs, as well as for those whose responsibility includes the teaching of psychiatry to student doctors, nurses and others.

JOHN L. HAMPSON

An Indexed Annual Review of the Progress of Ophthalmology. Indian Year-Book of Ophthalmology, 1954. Edited by VAMAN D. SATHAYE. 232 pp. Sangam Press, Poona, India.

One hundred and nineteen articles have been abstracted from three ophthalmological journals—The American Journal of Ophthalmology, The A.M.A. Archives of Ophthalmologp and The British Journal of Ophthalmology. In addition three short original articles have been included. Information about post-graduate courses in ophthalmology in different Indian Universities is given. Society proceedings of a number of ophthalmological societies are included. A list of eye specialists in India completes this volume.

There is little or no information in this volume that is not available elsewhere. There should be little demand to have this year-book perpetuated in its present form.

H.N.

Perspectives and Horizons in Microbiology. A symposium edited by SELMAN A. WAKSMAN. 220 pp., $3.50. Rutgers University Press, New Brunswick, N. J.

At Rutgers University on June 7, 1954, the dedication of the newly completed Institute of Microbiology was fittingly celebrated with a symposium by sixteen eminent microbiologists. Selman A. Waksman, first director of this Institute, edited their presentations for publication in this interesting volume.

Although the participants had been encouraged to peer ahead and give clues to future developments in the study of microscopic forms of life, a few frankly admitted the impossibility of prophecy, and others declined to comment on the future. The most sage observation, that of Joshua Lederberg, took note of the brashness such prediction would require before going on to point out that the significant concern of the future should be with questions we cannot presently formulate.

Microbiology studies all that is minute in life, and since the symposium was to indicate perspectives in microbiology, the range of topics was great. All discussants assumed both familiarity with the disciplines of microbiology and acquaintance with current thinking in this area of knowledge. The book is organized into four parts:

1) The Microbe as a Living System, 2) Metabolism of Microörganisms, 3) Microörganisms and Higher Forms of Life, 4) Appendix. Brief mention will be made of a few of the topics discussed.

The first section uses *the genetics of microörganisms* as its major vehicle for discussion. At first glance there seems to be little of medical interest. Indeed, in the first essay, Cornelius B. Van Niel makes it plain that microbiology no longer can be equated with medical and public health bacteriology and immunology. Further reading, however, causes one to reflect on the burgeoning evidence for biochemical universality amongst living organisms, regardless of size (for example, vitamin B12). Again, the *parasitization of bacteria* (discussed by André Lwoff) by viruses may seem remote from human concern, until one realizes there can occur far-reaching metabolic consequences as in the attainment of toxigenicity by *C. diphtheriae* on integration of suitable bacteriophage.

In the section dealing with the metabolism of microörganisms, Durey H. Peterson describes the remarkable ability of fungi and *bacteria as steroid chemists*. Microörganisms are capable of astounding synthetic feats, producing such compounds as cortisone, hydrocortisone, estrone, and testosterone if appropriate precursors are supplied.

In the third section, after contrasting the immense effort devoted to empiric search for antibiotics with the comparatively few studies on how antibiotics act on living cells, Harry Eagle discusses his own brilliant work on *the mode of action of penicillin. Chemical inhibition of viruses* (Frank L. Horsfall) is most efficient during that time of illness we know clinically as the incubation period. From this, one may conclude that prevention will remain the procedure of choice in viral diseases for some time to come.

Three dedicatory addresses make up the Appendix, and the volume ends on an historical note.

PAUL D. HOEPRICH

Pomp and Pestilence. Infectious Disease, Its Origins and Conquest. By Ronald Hare. 224 pp., $5.75. Philosophical Library, New York, N. Y.

The first two chapters of Professor Hare's exciting book illustrate what is meant by infection and how it is that man became the host for a variety of parasites responsible for disease. In the third chapter he discusses how epidemics of plague, typhus, smallpox, cholera, dysentery and influenza occurred and the influence these infections had on the history of sociological, economical, political and religious events. Chapter four shows chronologically, from primitive to recent times, man's evolving concepts and theorems of the causes of infectious disease. The following two chapters describe how communities and individuals assume importance in the control of parasitism. The elimination of epidemic disease from Western civilization contrasted with its persistence in other countries of the world is discussed in the last chapter. The world faces the problem of erasing epidemic infection from its remaining strongholds, but must propose solutions to overpopulation and starvation which may occur with the further control of parasitism.

This book beautifully describes how disease has influenced the course of history, and should be useful reading not only for those medically oriented.

LEIGHTON E. CLUFF

Bickham-Callander Surgery of the Alimentary Tract, Vols. I, II and III. By RICHARD T SHACKELFORD and assisted by HAMMOND J. DUGAN. 2575 pp., $60.00 per set. W.B. Saunders Co., Philadelphia, Pa.

This tremendous effort, almost entirely the work of a single author, is a complete revision of the old Bickham description of operative technics of general and special surgery. It accomplishes what the author sets out to do, namely to present nearly every operative procedure currently in use for repair of disease of the digestive tract. Perhaps this mission is accomplished too well as a number of procedures presented in detail seemed to this reviewer of historical interest only, but as such it is a ready and convenient reference work. Many of the technical descriptions are almost verbatim from articles of surgical authorities. The single authorship contributes significantly to the uniformity of style but loses some of the authoritative criticism most modern systems enjoy when written by many authors.

The book has a very pleasant format.

HENRY T. BAHNSON

BOOKS RECEIVED FOR REVIEW

The American Drug Index. By CHARLES O. WILSON and TONY EVERETT JONES. 576 pp., $5.00. J. B. Lippincott Co., Philadelphia, Pa.

The Biochemistry of Vitamin B_{12}. Biochemical Society Symposia No. 13. Edited by R. T. WILLIAMS. 123 pp., $3.75. Cambridge University Press, New York, N. Y.

Clinical Neurosurgery, Vol. II. Proceedings of the Congress of Neurological Surgeons, New York, N. Y. 1954. RAYMOND K. THOMPSON and IRA J. JACKSON, Co-editors in Chief. 173 pp., $6.75. The Williams & Wilkins Co., Baltimore, Md.

Die Röntgenbildanalyse. Eine Röntgendiagnostische Anleitung für Studierende und Ärzte. By ERICH SAUPE, with a foreword by W. TESCHENDORF. 283 pp., Ganzleinen DM 49.50 ($11.80). Georg Thieme Verlag, Stuttgart, Germany. Also Intercontinental Medical Book Cor., New York, N. Y.

The House Physician's Handbook. By C. ALLAN BIRCH. 160 pp., $3.00. E. & S. Livingstone, Ltd., Edinburgh and London. Distributed by The Williams & Wilkins Co., Baltimore, Md.

Hypnotic Suggestion. Its Role in Psychoneurotic and Psychosomatic Disorders. By S. J. VAN PELT. 95 pp., $2.75. Philosophical Library, New York, N. Y.

Surgery for Nurses, 3rd edition. By JAMES MORONEY. With a Foreword by DOROTHY M. SMITH. 696 pp., $6.00. E. & S. Livingstone, Ltd., Edinburgh and London. Distributed by The Williams & Wilkins Co., Baltimore, Md.

Therapy of Fungus Diseases. An International Symposium. Edited by THOMAS H. STERNBERG and VICTOR D. NEWCOMER. 337 pp., $7.50. Little, Brown and Co., Boston, Massachusetts.